Helsinki University Biomedical Dissertations No. CLXXVIII

Peutz-Jeghers Polyposis and the LKB1 Tumor Suppressor

Lina Udd

Molecular and Cancer Biology and Genome-Scale Biology,
Research Programs Unit &
Biochemistry and Developmental Biology,
Institute of Biomedicine,
Faculty of Medicine

and

Institute of Biotechnology

University of Helsinki
Finland

Academic Dissertation

To be publicly discussed with the permission of the
Faculty of Medicine, University of Helsinki
in Lecture Hall 2, Haartmaninkatu 3, Helsinki
on November 9th, 2012, at 12 noon.

HELSINKI 2012

Thesis supervisor

Tomi P. Mäkelä, M.D., Ph.D.
Institute of Biotechnology
University of Helsinki
Helsinki, Finland

Thesis follow-up committee

Ari P. Ristimäki, M.D., Ph.D.
Department of Pathology
HUSLAB and Haartman Institute
Helsinki University Central Hospital and University of Helsinki
Helsinki, Finland

Matti Airaksinen, M.D., Ph.D.
Neuroscience Center
Institute of Biotechnology
University of Helsinki
Helsinki, Finland

Reviewers appointed by the faculty

Pauli Puolakkainen, M.D., Ph.D.
Department of Gastroenterological Surgery
University of Helsinki and
Helsinki University Central Hospital
Helsinki, Finland

and

Tatiana Petrova, Ph.D.
Division of Experimental Oncology
CePO, CHUV and University of Lausanne
Lausanne, Switzerland

Opponent appointed by the faculty

Marnix Jansen M.D., Ph.D.
Department of Pathology
Academic Medical Centre
Amsterdam, The Netherlands

ISBN 978-952-10-8354-9 (paperback)
ISBN 978-952-10-8355-6 (PDF)
ISSN 1457-8433
http://ethesis.helsinki.fi
Unigrafia
Helsinki 2012

¡Hasta la victoria, siempre!

TABLE OF CONTENTS

ABBREVIATIONS

ACC = Acetyl-CoA carboxylase
Ad = Adenoviral
ADP = Adenosine diphosphate
Ah = Aryl hydrocarbon
ALK5 = Activin receptor-like kinase 5
AMPK = adenosine monophosphate (AMP) activated kinase
Apc/APC= Adenomatous polyposis coli
ARK5 = AMPK related kinase 5
ATM = Ataxia Telangiectasia mutated
ATG13 = Autophagy related 13 homolog
ATP = Adenosine triphosphate
BRSK = Brain-specific serine/threonine kinase
CAB39 = calcium binding protein 39
CaMKK = calcium/calmodulin dependent protein kinase kinase
Cdc37= Cell division cycle 37
CHIP = carboxyl terminus of Hsc70-interacting protein
COX-2 = Cyclooxygenase-2
CRM = Chromosome region maintenance
CT = Computer assisted Tomography
C-TAK = Cdc25 C-associated kinase
DCC = Deleted in colorectal cancer
DMBA = Dimethylbenzanthracene
Dn= dominant negative
4E-BP= Eukaryotic translation initiation factor 4E-binding protein
EMT= epithelial-to-mesenchymal transition
ERK= Extracellular-signal-regulated kinases
ERT= Estrogen receptor Tamoxifen inducible
FAP= Familial Adomatous Polyposis
FIP200= focal adhesion kinase family interacting protein of 200 kD
FH= Fumarate hydratase protein
GSK= Glycogen synthase kinase
HDAC4= Histone deacetylase 4
Het= heterozygous
HIF= Hypoxia inducible factor
HLRCC = Hereditary leiomyomatosis and renal cell cancer
Hmz= homozygous
HPGL= hereditary paragangliomatosis with phaeochromocytomas
Hsp= heat shock protein
i.p.= intraperitoneal
IP = immunoprecipitate
KD = kinase dead
KLK10= kallikrein-related peptidase 10
LKB1= Liver Kinase B1
LOH= Loss of heterozygosity
LOX= Lysyl Oxidase
MAF1= Mouse Repressor of RNA polymerase III transcription
MARK= Microtubule affinity-regulating kinase

MAPs= Microtubule associated proteins
MLC= Myosin light chain
mLST8= Mammalian target of rapamycin (mTOR) associated protein
Mx1= Myxovirus resistance 1
LST8 homolog
MEF= mouse embryonic fibroblast
MEK= MAPK/Erk kinase
Misr2= Muellerian inhibiting hormone receptor 2
Mo25= Mouse protein 25
MVD= Microvessel density
mTOR= Mammalian target of rapamycin
NADPH= Nicotinamide adenine dinucleotide phosphate
NES= nuclear export signal
NF= Neurofibromin proteins
NLS= nuclear localization signal
NUAK= Nuclear AMPK-related kinase
PanIn= Pancreatic Intraepithelial Neoplasia
PAPG= Pepsinogen altered pyloric gland
Par-1= Partitioning defective 1
PCR= polymerase chain reaction
Pdx1= Pancreatic and duodenal homeobox 1
PI3K=phosphoinositide-3-kinase
PINK1= Phosphatase and tensin homolog (PTEN) induced kinase 1
PIP3= phosphatidylinositol-trisphosphate
pIpC= polyinosinic-polycytidylic acid
PJS= Peutz-Jeghers Syndrome
PKA= Protein kinase A
PKB= Protein kinase B
PKC(zeta)=Protein kinase Cζ
POMC= Pro-opimelanocortin
Ppm= parts per million
PRKC= Protein kinase C
PSCD2= pleckstrin homology, Sec7 and coiled-coil domains 2
PTEN= Phosphatase and tensin homolog
pVHL= Von Hippel-Lindau protein
RAF= Rapidly accelerated fibrosarcoma proteins
RAS= Rat sarcoma proteins
Rheb= Rat sarcoma protein (Ras) homolog enriched in brain
Rip2= Receptor interacting protein 2
RSK= p90 ribosomal S6 kinase
RTKs= receptor tyrosin kinases
SAD= Synapses-of-amphids-defective kinase
SCTAT= sex-cord tumor with annular tubules
SDH= Succinate dehydrogenase protein
Sdr.= Syndrome
SIK= Salt inducible kinase
S6K= p70 ribosomal S6 kinase
SMAD2= Mothers against decapentaplegic homolog 2
SNARK= sucrose non-fermenting AMPK-related kinase
SNRK= sucrose non-fermenting (SNF) related kinase

Spdef= Sam pointed domain ETS factor
SRC= sarcoma protein
STK= Serine/threonine kinase
STRAD= Ste20-related kinase adapter protein
TAK-1= TGFβ activated kinase 1
TCA-cycle= Tricarboxylic acid cycle
TFF2= Trefoil factor 2
TG= transgenic
TGFβ= Transforming growth factor β
TORC= Transducer of regulated cAMP-response-element-binding (CREB)-binding protein
TSC= Tuberous sclerosis proteins
TUNEL= Terminal transferase dUTP nick end labeling
Ubc= Ubiquitin
ULK1/2= unc-51-like kinase
VEGF= Vascular endothelial growth factor
WEF=Trp-Glu–Phe
Wnt= Wingless and activator of Integration1
wt= wildtype

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications (I-III) and a manuscript (IV), which are referred to in the text by their Roman numerals.

I LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including, MARK/PAR-1. Lizcano, J. M, Goransson, O., Toth, R. Deak, M., Morrice, N. A., Boudeau, J., Hawley, S. A., Udd, L., Makela, T. P., Hardie, D. G., Alessi, D. R., Embo J, 2004, 23, 833-43.

II Suppression of Peutz-Jeghers polyposis by inhibition of cyclooxygenase-2. Udd L, Katajisto P, Rossi DJ, Lepistö A, Lahesmaa AM, Ylikorkala A, Järvinen HJ, Ristimäki AP, Mäkelä TP. Gastroenterology. 2004 Oct;127(4):1030-7.

III Impaired gastric gland differentiation in Peutz-Jeghers syndrome. Udd L, Katajisto P, Kyyrönen M, Ristimäki AP, Mäkelä TP. Am J Pathol. 2010 May;176(5):2467-76.

IV Increased sensitivity to N-Methylnitrosourea induced DNA damage in *Lkb1*$^{+/-}$ mice, Udd L, Ristimäki AP, Mäkelä TP, submitted.

Author's contributions:

I *Planned and performed the generation of E9.5 Mouse embryonic cell lines; wrote the manuscript section regarding this procedure.*

II *Participated in planning the study setup, filed for ethical permissions and performed collaborative correspondence, performed and analyzed the mouse experimentation, participated in the analysis of patient data, main writer of the manuscript except regarding patient data. Publication also included in thesis of Dr. Pekka Katajisto.*

III *Planned the study setup, filed for ethical permissions and performed collaborative correspondence, planned and performed and analyzed or planned and analyzed all experimentation except the microarray experiment, main writer of the manuscript except regarding microarray data.*

IV *Planned the study setup, filed for ethical permissions and performed collaborative correspondence, planned and performed and analyzed all experimentation, main writer of the manuscript.*

ABSTRACT

The Peutz-Jeghers Syndrome is a rare cancer predisposition condition, caused by mutations inactivating the LKB1 tumor suppressor kinase. This study aimed to further the understanding of this disease, provide potential means of treatment to Peutz-Jeghers patients, and to add to our understanding of cancer formation in general. These aims were pursued through exploring the molecular functions of the LKB1 kinase, studying the tumor formation upon loss of LKB1 function, and through intervening with this tumor formation process. The study was mainly performed in the *Lkb1* knockout mouse or derived tissues and cells, but partly also with Peutz-Jeghers patients and patient materials.

We found that the LKB1 kinase phosphorylates and thereby activates 13 kinases in the AMP-activated kinase family, any of which could putatively relay the tumor suppressor functions of LKB1. We also found that Cyclooxygenase-2 participates in tumorigenesis in Peutz-Jeghers syndrome by promoting the growth of gastric polyps, and that inhibitor treatment suppresses the polyp formation. We also observed that these Peutz-Jeghers polyps are less differentiated than previously thought, and that signs of poor differentiation can be seen in the gastric epithelium already prior to polyp formation. In addition, we found that the polyp formation process is likely to be enhanced by other genes in addition to *LKB1* and *Cycloxygenase-2*, as alkylating mutagenesis increased polyp formation independently of the activity of the latter.

Taken together, these results point to a wide array of molecules and processes interplaying in Peutz-Jeghers tumorigenesis beyond the LKB1 kinase. Both straight molecular targets of LKB1 activity, indirect mediators of LKB1-regulated tumorigenesis, and cooperating processes have been identified. One may expect that these findings will be of use for future studies both characterizing the Peutz-Jeghers syndrome and targeting treatments for this and related tumor diseases.

INTRODUCTION

Cancer is a worldwide concern, affecting more than every third person in developed countries (www.cancer.org and info.cancerresearchuk.org). It is caused by mutations affecting the growth of cells and how they interact with their surroundings. Such mutations usually occur by environmental impact, although some mutations predisposing for cancer are inheritable and passed on in families as disease entities. The Peutz-Jeghers syndrome (PJS) discussed below in this study is an example of such an inheritable condition, although in addition to being inherited (as an autosomal dominant disease) it may also occur sporadically by de novo mutation.

The frequency estimates for PJS lie somewhere between 1:29 000 (Mallory and Stough 1987) to less than 1:200 000 (Eng et al. 2001) rendering it very rare in comparison to the worldwide cancer burden. On the other hand, being a genetically characterized disease, dependent on a single gene, *Liver Kinase B1* (*LKB1*), PJS has offered a route to model and explore cancer from the perspective of this particular tumor suppressor. Study tools and lessons learnt from the experimental models of PJS have also been used in the study of cancer beyond the Peutz-Jeghers syndrome (as discussed further below).

REVIEW OF THE LITERATURE

1 The Peutz-Jeghers syndrome

The Peutz-Jeghers Syndrome (PJS) presents as triad of mucocutaneous pigmentations, polyposis and increased cancer risk (Peutz 1921; Jeghers et al. 1949). The malignant tumors are mainly carcinomas and most commonly arise in the gastrointestinal tract, where the relative risks of developing cancer has been estimated to be 84-fold for colon, 213-fold for gastric, and 520-fold for small intestinal cancer (Giardiello et al. 2000).

A number of other cancer types, like pancreatic, breast and lung cancers also occur with a much higher frequency in PJS than in the normal population(Hearle et al. 2006a; van Lier et al. 2011), and some very unusual cancer types occur, including adenoma malignum of the cervix, Sertoli cell tumour of the ovary and feminizing Sertoli cell tumor of the testes. Also, the ovarian sex-cord tumors with annular tubules (SCTAT), which only rarely have been observed in non-PJS patients, deserve to be mentioned as a feature of this disease.

The Peutz-Jeghers polyps are characterized by a stroma with abundant smooth muscle forming branching bundles (Rintala 1959) and occur throughout the gastrointestinal tract

(stomach, small intestine, colon and rectum), and in some patients also in other locations like the nose or gallbladder (Wada et al. 1987; de Leng et al. 2007a) and in single cases also the urinary tracts (Sommerhaug and Mason 1970), and bronchi (Sommerhaug and Mason 1970). They are benign hamartomatous tumors, and as discussed further below, it is unclear whether the gastrointestinal carcinomas originate from polyps or randomly, rendering the two as completely distinct entities. Despite this benign nature, the polyps cause serious complications to the Peutz-Jeghers patients if left uncontrolled, as they may bleed, obstruct or intussuscept the intestine.

Point mutations affecting the *LKB1(STK11)* gene are known to cause PJS in around 70% of cases, and large deletions of the whole *LKB1* locus on chromosome 19p13.3 may raise the number of cases explained by *LKB1* mutations to >90% (Aretz et al. 2005; Hearle et al. 2006b; Volikos et al. 2006). Other genes that would cause PJS have not been found, despite efforts screening genes such as the homeobox gene *CDX2*, *serine-threonine kinase 13* (*STK13*), *Protein-kinase C gamma* (*PRKCγ*), the *kallikrein-related peptidase KLK10*, *Pleckstrin homology, Sec7 and coiled/coil domains 2 gene* (*PSCD2*), the LKB1-interacting proteins *STK11IP*, *BRG1*, *STRADα*, and *MO25α*, as well as the polarity-associated *MARK/Par1* gene family (Woodford-Richens et al. 2001; Buchet-Poyau et al. 2002; Alhopuro et al. 2005; de Leng et al. 2007b).

1.1 Peutz-Jeghers polyposis among the gastrointestinal polyposes

Gastrointestinal polyps can be classified into neoplastic (like adenomatous, serrated and carcinoid polyps), hyperplastic (including inflammatory polyps), hamartomatous (e.g. Peutz-Jeghers, fundic-gland, juvenile, and Cowden polyps) and mesenchymal (gastrointestinal stromal tumors, smooth muscle, neural, lymphoid or vascular tumors) types.

Adenomatous polyps in particular have been extensively studied, due to their clinical significance being both common and pre-malignant, and a detailed model of the morphological and molecular chain of events leading to their formation and progression was presented already two decades ago (Fearon and Vogelstein 1990). In this model, a cell in the normal epithelium adquires two mutations in the *Adenomatous Polyposis Coli* (*APC*) gene, turning its progeny epithelium dysplastic. Hypomethylation changes the expression patterns in this epithelium turning it into an early adenoma, then *K-Ras* mutations and later *SMAD2/DPC4/DCC* mutations occur as the adenoma progresses becoming an intermediate and then a late adenoma. As *p53* mutations occur, the adenoma progresses into an in situ carcinoma, which further develops into an invasive carcinoma. As *SRC* gene mutations occur the carcinoma is ready to metastasize.

Peutz-Jeghers polyps, as well as other more rare polyps, have naturally been analyzed for the markers already characterized in adenoma-carcinoma progression, like the loss of tumor suppressor functions in the epithelium, and Wnt-signaling deregulation. In PJS polyposis aberrant Wnt signaling has been implicated to arise through failure of mutated LKB1 to activate Wnt signaling inhibitor GSK-3β, and through overexpression of Wnt5a (Lai et al. 2011). In immunohistochemical studies, β-catenin dislocation (Chaiyapan et al. 2010; Ma et al. 2010) and focal p53 overexpression (Entius et al. 2001) have been observed in PJS patient polyps.

However, unlike in the adenomas or, for instance, the hamartomatous polyps of Juvenile Polyposis (Woodford-Richens et al. 2000), there is no evidence that the epithelium of Peutz-Jeghers polyps would represent a clonal expansion, rather, the Peutz-Jeghers polyp epithelium is polyclonal and presents with hyperproliferative but otherwise correctly organized and separated epithelial units (de Leng et al. 2007c). Thus, the Peutz-Jeghers polyp initiation and progression differs dramatically from the adenoma model, and although it does not exclude focal bursts of clonal expansion and adenoma-carcinoma progression within the polyp (Gruber et al. 1998), it does not imply that such progression would inevitably occur in PJS polyps, or that such clonal expansions would be more probable within a polyp than in the mucosa next to it.

2. The LKB1 kinase

LKB1 is a serine-threonine kinase. It reaches its full kinase activity only when in a complex with two other proteins, STRAD (LYK5) and Mo25 (CAB39) (Zeqiraj et al. 2009), which also stabilize the LKB1 protein and keep it in the cytoplasm, where it can phosphorylate its substrates (Figure1, (Dorfman and Macara 2008)).

LKB1 also associates with the molecular chaperone heat shock protein 90 (Hsp90) and Cdc37, which stabilize LKB1. A balance between chaperone binding and degradation was recently suggested to regulate the levels of LKB1 activity in the cell. LKB1 kinase activity was found to be transiently stimulated upon dissociation of Hsp90, which simultaneously caused recruitment of Hsp/Hsc70 and the ubiquitin ligase CHIP (carboxyl terminus of Hsc70-interacting protein) triggering subsequent LKB1 degradation (Boudeau et al. 2003a; Gaude et al. 2012).

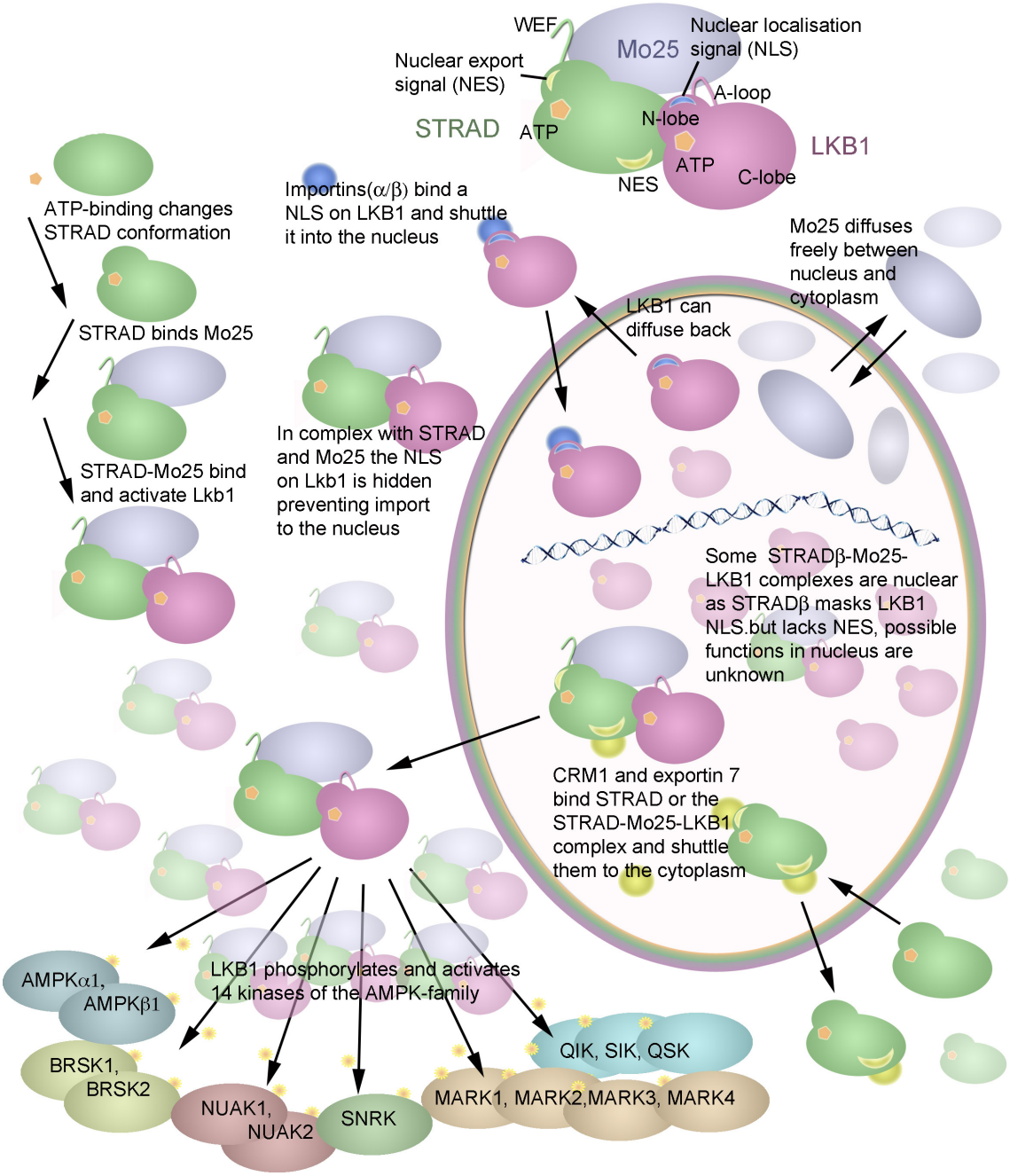

Figure 1. Regulation of LKB1 activity. A schematic depiction of the inside of a cell, with the nucleus on the right. The known kinase activities of LKB1 take place in the cytoplasm (lower left-hand corner). LKB1 possesses a nuclear localization signal (NLS, upper right-hand corner). Without its complex binding partners Mouse protein 25 (Mo25) and Ste20-related kinase adapter protein (STRAD), LKB1 is almost inactive, and the NLS is exposed to importins, which shuttle it into the nucleus (center of picture). The STRAD-Mo25-complex binds to LKB1 in a serial order (upper left-hand corner), with Adenosine triphosphate (ATP) binding triggering LKB1 binding, and a Trp-Glu –Phe (WEF) motif on STRAD triggering Mo25 binding. The main form of STRAD, STRADα, has two nuclear export signals (NES) tagging it (or the whole complex) for shuttle to the cytoplasm by Chromosome region maintenance (CRM) protein 1 or Exportin 7(lower right corner). STRADβ, on the other hand, does not contain NESs, leaving some active LKB1 complexes inside the nucleus. These nuclear LKB1 complexes have no currently known function. The schematic is modified from (Dorfman and Macara 2008) with additional data from (Lizcano et al. 2004; Jaleel et al. 2005; Zeqiraj et al. 2009a; Zeqiraj et al. 2009b).

PJS-causing mutations either affect the LKB1 kinase function directly by disrupting the kinase domain, or indirectly by affecting the binding to this complex (Boudeau et al. 2004). Among the other LKB1 complex proteins, Mo25 is a chaperone regulating several different kinase complexes (Boudeau et al. 2003b; Filippi et al. 2011), and STRAD is a pseudokinase, which binds ATP, but has no own kinase activity (Baas et al. 2003). Mutations in STRAD have been found to cause another inherited disease, the polyhydramnios, megalencephaly and symptomatic epilepsy (PMSE) syndrome (Puffenberger et al. 2007), through impaired corticogenesis (Orlova et al. 2010).

LKB1 also undergoes several posttranslational modifications (Figure 2) to reach its full activity, multiple phosphorylations by different kinases including Protein kinase Cζ (Xie et al. 2009), p90 ribosomal protein S6 kinase (RSK) (Sapkota et al. 2001) and Protein kinase A (PKA), as well as prenylation (farnesylation) (Collins et al. 2000).

There are two different splice variants of LKB1, a full length, major form, and a shorter version denoted LKB1s, predominantly expressed in testis(Denison et al. 2009; Towler et al. 2008). The regulatory complex partners present with two different gene isoforms each, STRADα STRADβ, Mo25α and Mo25β. STRADα, the main isoform of STRAD has also been described to have 11 different splice variants, of which only STRADα-1 and STRADα-2 are able to form a complex with Mo25 and LKB1(Marignani et al. 2007).

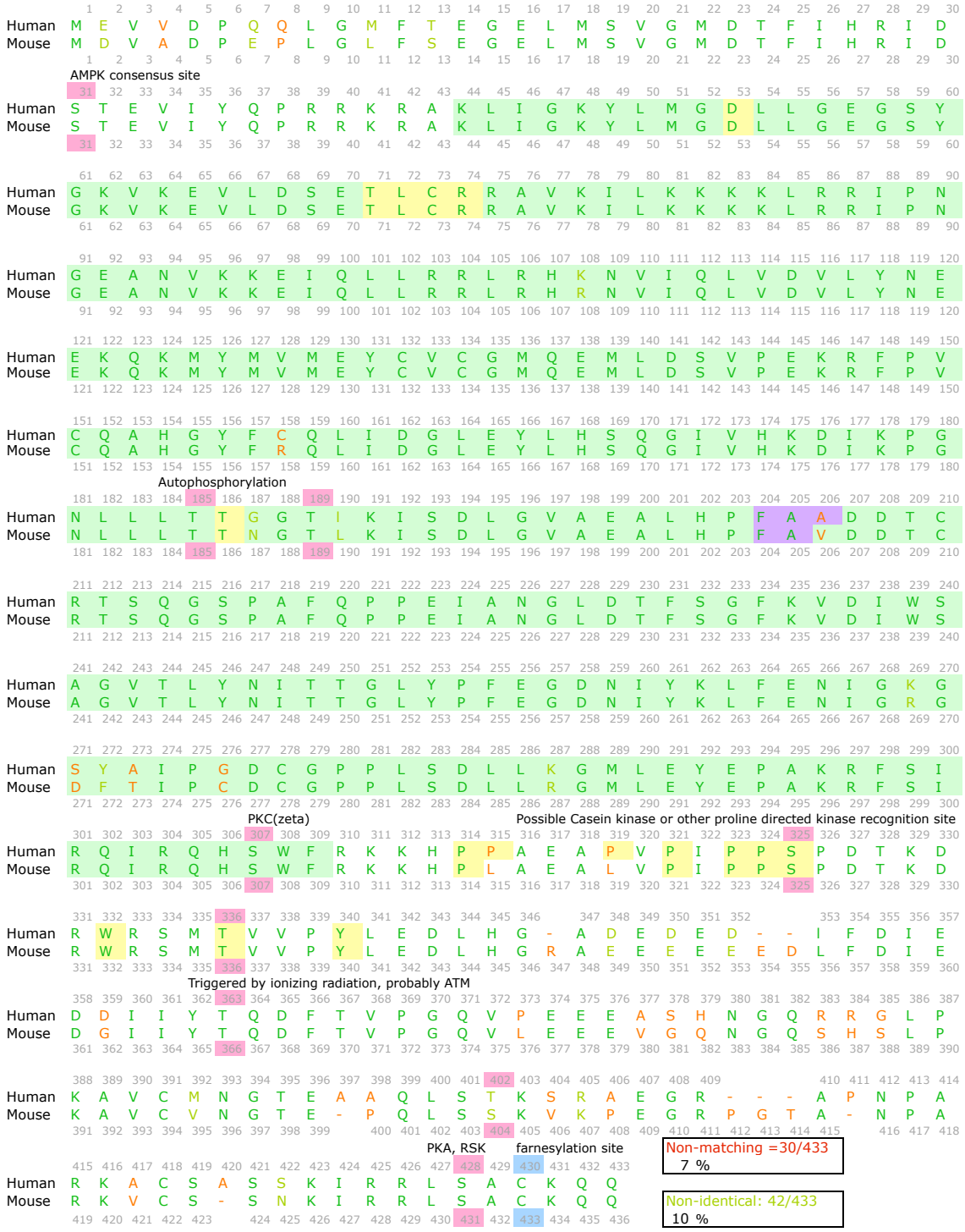

Figure 2. Comparison of human LKB1 and mouse Lkb1. The alignment demonstrates the high conservation of LKB1 and Lkb1 with 90% identity (93% similarity). Coloring indicates the kinase domain (green), residues important for MO25 binding (purple), STRAD binding (yellow), phosphorylation (pink) and prenylation (blue). Kinases implicated in some of the phosphorylations are indicated above the alignment. AMPK = Adenosine monophosphate activated kinase, ATM= Ataxia Telangiectasia mutated, PKA= Protein kinase A, PKC(zeta)=Protein kinase Cζ, RSK= p90 ribosomal protein S6 kinase. Figure based on a BlastP search query, with additional information from (Wera 1999; Collins et al. 2000; Sapkota et al. 2001; Sapkota et al. 2002; Martin and St Johnston 2003; Shaw et al. 2004a; Xie et al. 2009; Zeqiraj et al. 2009).

3. LKB1-mediated cellular functions

All known LKB1 substrates are kinases in the Adenosine monophosphate activated kinase (AMPK) family (Lizcano et al. 2004; Jaleel et al. 2005). Phosphorylation by LKB1 causes activation of the substrates (I), which in turn regulate a wide array of cellular processes involving the physical structures, metabolic states as well as the external signaling of the cells (Figure 3, and discussed below). LKB1 also associates with the transcription activator and ATP-dependent helicase BRG1 without phosphorylating it (Marignani et al. 2001), but still increasing the activity of this helicase.

New aspects of this potentially LKB1-regulated signaling network of AMPK and the AMPK related kinases keep emerging, with varying conclusions drawn from different cell types and different settings. As a general rule, though, we have proposed that, independently of tissue type, the net effect of LKB1 signaling is to advance cellular differentiation, as this end result has been shown in enterocytes, gastrointestinal secretory cells, exocrine and endocrine pancreatic cells, brown adipocytes, myofibroblasts, neuronal cells, lymphoid B- and T-cells as well as germinal cells (Udd and Makela 2011).

Advancing cellular differentiation is a property of many other tumor suppressors as well, like p53 (Lin et al. 2005), the Retinoblastoma protein (Zacksenhaus et al. 1996), Neurofibromin-1 (Hegedus et al. 2007), or the Wilms tumor suppressor 1 (Ellisen et al. 2001).

3.1 Functions of the AMP-activated Kinase

The AMP-activated Kinase (AMPK), consists of three subunits, α, β and γ, with the kinase domain residing in α. There are several isoform genes for each subunit, (α1, α2, β1, β2, γ1, γ2, γ3), and the γ2 and γ3 genes have splice variants. All unit types can form complexes with each other *in vitro*, but the composition of the complexes present *in vivo* is dependent both on tissue type and intracellular location (Viollet et al. 2009a).

AMPK, as its name indicates, is activated by a lowered energy potential inside the cell reflected in an increase in monophosphorylated adenosines (AMP) which allosterically regulate its kinase activity severalfold (Carling et al. 1989). Phosphorylation by LKB1, in comparison, activates AMPK several hundredfold (Hawley et al. 2003). Allosteric regulation by either AMP or ADP also potentiates phosphorylation mediated activation as it prevents dephosphorylation of AMPK, although ADP in itself does not activate AMPK (Davies et al. 1995; Xiao et al. 2011). Apart from LKB1, the Ca^{2+}/calmodulin-dependent protein kinase kinaseβ (CaMKKβ, important at least in brain) and by TGFβ

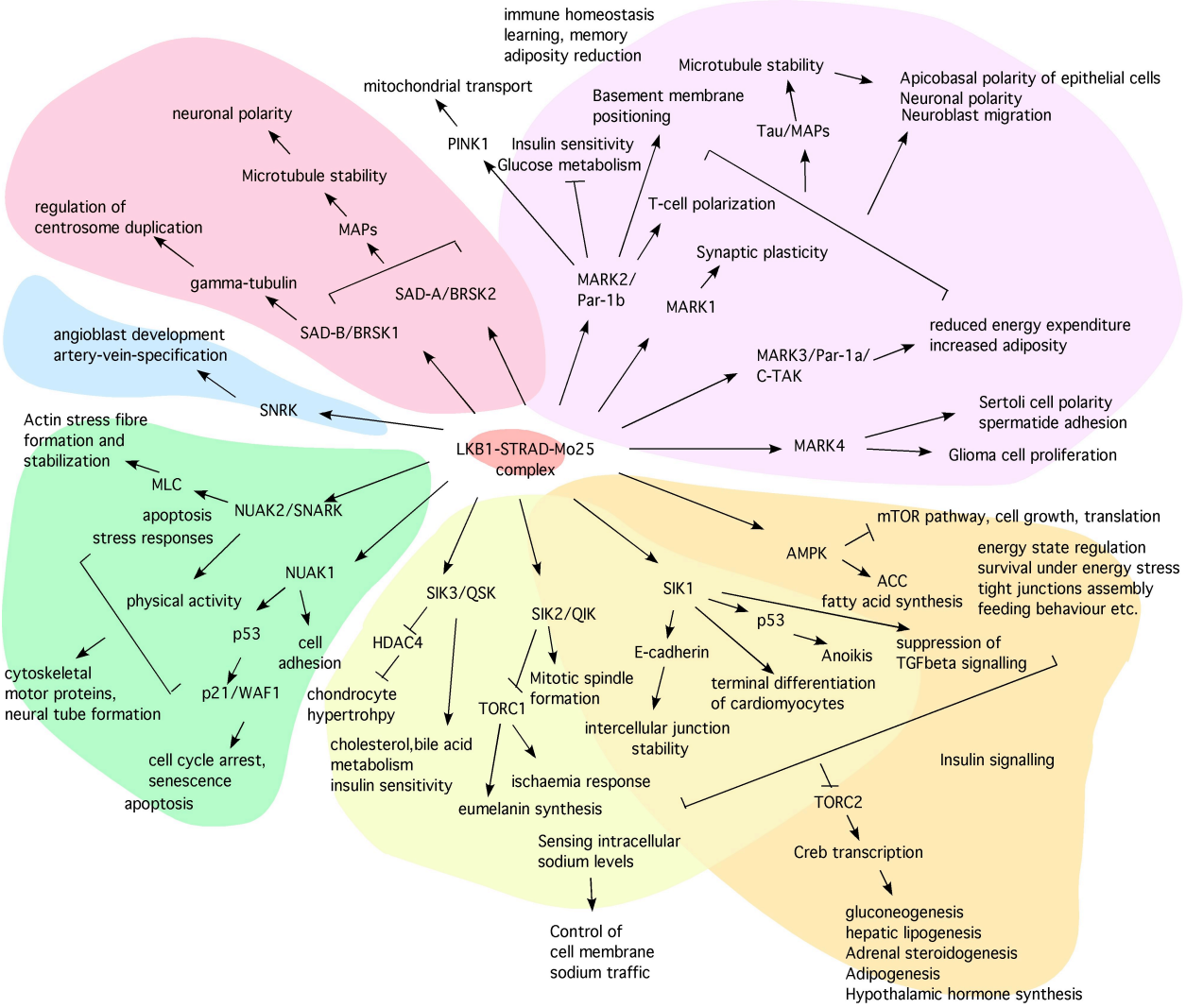

Figure 3. Signaling of AMPK-related kinases. The LKB1 substrates belonging to the AMPK family regulate a wide array of cellular processes, positioning LKB1 as a potential regulator of these processes. In this map, a selection of pathways and processes regulated by the AMPK related kinases are presented, to illustrate the width of the spectrum of validated and potential LKB1 functions. When required, the LKB1 substrates are labeled multiply to visualize their complex nomenclature. Arrows indicate activation of the respective target, blunt ends indicate suppression. Processes peripheral to brackets are affected by all LKB1 substrate kinases within the bracket span. For gene product abbreviations explained please see the List of Abbreviations above. Adapted from (Katajisto et al. 2008) with additions from (Beghini et al. 2003; Barnes et al. 2007; Kowanetz et al. 2008; Alvarado-Kristensson et al. 2009; Bright et al. 2009; Chun et al. 2009; Ichinoseki-Sekine et al. 2009; Horike et al. 2010; Koh et al. 2010; Lennerz et al. 2010; Romito et al. 2010; Hou et al. 2011; Klutho et al. 2011; Sasaki et al. 2011; Vallenius et al. 2011; Daley et al. 2012; Eneling et al. 2012a; Eneling et al. 2012b; Liu et al. 2012; Matenia et al. 2012; Ohmura et al. 2012; Sasagawa et al. 2012; Tang et al. 2012; Uebi et al. 2012)

activated kinase (TAK1, no physiological context described yet), can also phosphorylate and activate AMPK (Bright et al. 2009).

AMPK has an important role as a regulator of energy-dependent cell methabolism. Upon energy stress, AMPK activation suppresses the mTOR pathway, which is active under nutrient rich conditions and enhanced by growth factor signaling (Shackelford and Shaw 2009) thereby limiting anabolic processes in the cell and ultimately also cell growth. AMPK also directly inhibits fatty acid synthesis by phosphorylating ACC1 (Hardie and Carling 1997) and increases "recycling" of nutrients through autophagy, by associating with and phosphorylating Unc-51-like kinase (ULK1) (Egan et al. 2011). AMPK activity also seems necessary for cell polarity and mitotic progression, at least in drosophila (Lee et al. 2007) and it has anti-inflammatory properties, demonstrated e.g. by Buler et al. (Buler et al. 2011).

Total knockout of AMPK activity through deletion of both genes for kinase subunits ($\alpha1^{-/-}$ and $\alpha2^{-/-}$ double knockout animals) is embryonic lethal at ~E10.5. $AMPK\alpha1^{-/-}$ mice have no metabolic phenotype whereas $\alpha2^{-/-}$ mice do, and thus the latter kinase subunit has been more extensively studied. (Viollet et al. 2003; Viollet et al. 2009a). In addition, tissue specific transgenic and knockout mice have been produced to examine the roles of the different AMPK subunits in metabolically active tissues like the liver, where e.g. $\alpha1^{-/-}$ and $\alpha2^{-/-}$ double knockout leads to reduced mitochondrial biogenesis (Guigas et al. 2007), and AMPKα2 has been found (both through knockout and overexpression studies) to suppress triglyceride release and increase ketone body formation(Foretz et al. 2005; Andreelli et al. 2006) or heart, where the glycolytic response to ischaemia is impaired e.g. upon overexpression of dominant-negative AMPKα2 (AMPKα2-KD)(Russell et al. 2004).

3.2 Functions of the AMPK related kinases

The functions of the other kinases of the AMPK family do overlap to some extent with those of AMPK itself, however they are also much more diverse (Figure 3), and whether these kinases also can be phosphorylated by CaMKKβ or TAK1 is unclear (Bright et al. 2009). The BRSK/SAD kinases are necessary for neuronal cell polarization and axon formation (Barnes et al. 2007), and a short splice variant of SADB seems to have a role in the control of centrosome duplication in all cells types, via phosphorylation of γ-tubulin(Alvarado-Kristensson et al. 2009).

The Par-1 family or microtubule associated protein regulating kinases (MARKs) were originally characterized as regulators of oocyte and epithelial cell polarity through

regulating microtubule stability. The MARKs phosphorylate microtubule-associated proteins such as tau (which forms the aggregates seen in Alzheimers disease). Mouse studies of MARK2 and MARK3 knockout have rather revealed that these kinases compensate for each other during embryogenesis (double knockout being embryoniclethal), but cause very disparate, metabolic phenotypes in the adult. Increased adiposity, insulin hypersensitivity, and aberrant glucose metabolism for MARK3 (Lennerz et al. 2010) and increased insulin sensitivity, increased glucose tolerance, and resistance to diet-induced obesity for MARK2. (Klutho et al. 2011; Bessone et al. 1999; Hurov et al. 2007)

Studies of MARK2 function on a cellular level has revealed more structural/polarity-related details, such as being required for correct positioning of the basement membrane of epithelial surfaces(Daley et al. 2012) or promotion of mitochondrial transport within neurons (Matenia et al. 2012). Testis-specific adherens junctions are maintained by MARK4, whose disruption leads to detachment of spermatids from the Sertoli cells(Tang et al. 2012). Glioma cell proliferation is enhanced by amplification and overexpression of MARK4(Beghini et al. 2003) implicating it as a tumorigenic kinase rather than possessing a tumor tuppressor function.

The salt inducible kinases (SIK1-3), as the name indicates, are activated by an increased Na^+ concentration and activation of the plasma membrane pumps for sodium ion exchange. Among these, SIK1 has been found to actively increase the transport of sodium in response to increased Na^+ concentration and adrenergic stimuli (Eneling et al. 2012b). In lung alveolar cells, LKB1 has been found to regulate E-cadherin expression and the stability of intercellular junctions through SIK1(Eneling et al. 2012a). SIK1 is also an inhibitory modulator of the cellular response to TGFβ, as it causes degradation of activated ALK5 recepors(Kowanetz et al. 2008). SIK1 also specifically regulates the differentiation of embryonic stem cells into cardiomyocytes. (Romito et al. 2010).

Both SIK1 and SIK2 have been shown to be involved in the regulation of corticotropin-releasing hormone transcription in the hypothalamus, with SIK2 mediating TORC inactivation in basal conditions, and induction of SIK1 limiting activation responses in its transcription(Liu et al. 2012). SIK2 specifically suppresses eumelanogenesis, as Agouti mice regained a brown hair color when crossed with *SIK2*-deficient mice (Horike et al. 2010). These *SIK2* knockout mice also had an altered response to cerebral ischaemia, with improved neuronal survival(Sasaki et al. 2011). SIK3, on the other hand, has been found necessary for chondrocyte hypertrophy during endochondral ossification occurring in growing long bones in mice(Sasagawa et al. 2012). *SIK3* knockout mice also show hypolipidemia, hypoglycemia, and increased insulin sensitivity due to aberrant fatty acid, cholesterol and bile acid metabolism in the liver (Uebi et al. 2012).

Whereas SIK1 is implicated in p53 dependent cell cycle arrest and senescence (anoikis) (Cheng et al. 2009), Nuak1 has been found to mediate LKB1 dependent p53 activation and apoptosis, at least *in vitro* (Hou et al. 2011). NUAK2 activity is increased in skeletal muscles upon contraction, and the muscles of *NUAK2*-heterozygous mice show impaired contraction-stimulated glucose transport (Koh et al. 2010). NUAK2 is also important for the contractile properties of nonmuscle cells by activating the formaton of stress fibres (Vallenius et al. 2011). Surprisingly however, although *NUAK2*-heterozygous mice collect more adipose tissue when sedentary, they are more active than wildtype mice when offered a possibility to exercise (Ichinoseki-Sekine et al. 2009). Embryonic double mutants of *NUAK1* and *NUAK2*, on the other hand, show neurodevelopmental defects, like exencephaly, facial clefting and spina bifida (Ohmura et al. 2012). Sucrose non-fermenting related kinase 1 (SNRK1), a kinase more distantly related to AMPK, and also a member of its own SNRK family, has a role in the migration of angioblasts during artery-vein specification, as shown in zebrafish (Chun et al. 2009).

4. From loss of LKB1 signaling to Peutz-Jeghers Symptoms

The insights into the molecular pathways governed by LKB1, have linked PJS with the molecular events underlying other hamartoma syndromes (Figure 4), through the mTOR pathway (van Veelen et al. 2011) and Hypoxia-inducible factor (HIF)-1α (Brugarolas and Kaelin 2004)(Figure 4), which are indirectly suppressed by AMPK activation (Corradetti et al. 2004). Whether this pathway is the key to the characteristic symptoms of PJS or whether disruption of other LKB1 substrate functions play a role remains under debate. Indeed, the PJS polyps have been suggested to arise through mTOR pathway dysregulation (Shaw et al. 2004b; Shackelford et al. 2009), but also, potentially through defects in epithelial polarity regulating pathways through the MARKs (Par-1) (Baas et al. 2004; Jansen et al. 2006). The lentigines, on the other hand, have been suggested to arise through deficient SIK2-signaling in melanocytes, although this has not been directly demonstrated (Horike et al. 2010).

The sets of LKB1 effectors, however, depend both on tissue type and developmental stage (Figure 3, as well as mouse models of Lkb1 loss described below and in Tables 1-3), and LKB1 actually possesses all categories of tumor suppression function (Kinzler and Vogelstein 1998). It is both a caretaker enhancing cellular differentiation (as discussed above in Chapter 3 and (Udd and Makela 2011)), a gatekeeper, through direct control of the cell cycle (Tiainen et al. 2002) and apoptosis (Karuman et al. 2001; Lee et al. 2006), and a landscaper, balancing the stromal-epithelial crosstalk (Katajisto et al. 2008; Tanwar et al. 2012). This suggests, that the mechanisms behind the PJS symptoms will turn out more complex, rather than all driven by mTOR pathway activation.

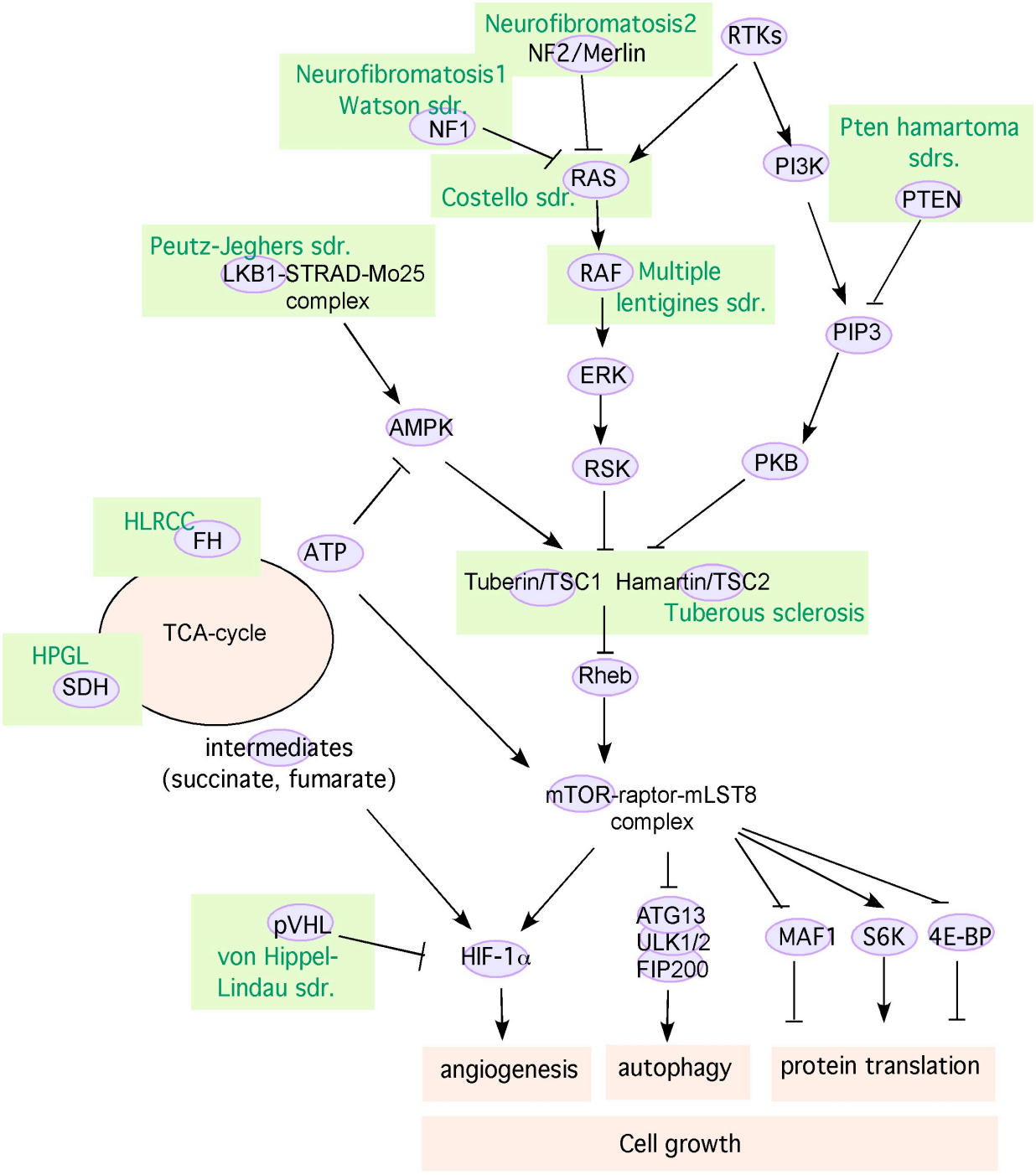

Figure 4. Connections between the hamartoma syndromes. A large group of inherited syndromes (green) presenting with hamartomatous tumors, cancers and mucocutaneous pigmentation defects link together through signaling connections by gene products (purple) implicated in their pathogenesis molecularly. Some converge on activation of the Ras signaling pathway, others join in on an activated mTOR pathway, and all display activated HIF1-α signaling. Arrows indicate activation of the respective target, blunt ends indicate suppression. Pten related Hamartoma syndromes include the Cowden syndrome, Bannayan-Riley-Ruvlcaba syndrome, PTEN-related Proteus syndrome and Proteus-like syndrome. HLRCC = Hereditary leiomyomatosis and renal cell cancer, HPGL= hereditary paragangliomatosis with phaeochromocytomas. TCA-cycle = citric acid cycle. For gene product abbreviations please see the List of Abbreviations above. Adapted from (van Veelen et al. 2011), with additions from (Pollard et al. 2005; Koivunen et al. 2007; Reig et al. 2011).

5. Loss of *LKB1* in cancer beyond the Peutz-Jeghers Syndrome

Early studies of *LKB1* in sporadic cancers showed little success in finding roles for *LKB1* beyond the Peutz-Jeghers syndrome (Avizienyte et al. 1998; Bignell et al. 1998; Avizienyte et al. 1999), with the exception of a 5% occurrence of biallelic inactivating mutations of *LKB1* seen in pancreatic and biliary adenocarcinomas (Su et al. 1999). A breakthrough on this point came when *LKB1* inactivation was identified as a frequent event in non small-cell lung cancer (Sanchez-Cespedes et al. 2002). This observation was followed by similar observations in breast (Shen et al. 2002; Yang et al. 2004) and cervical cancer (Wingo et al. 2009). Curiously, in breast cancer, inactivation often occurs through methylation of *LKB1* rather than through gene alterations (Fenton et al. 2006), suggesting also other forms of cancer may need to be revisited to clarify whether *LKB1* function is maintained.

6. Modeling loss of *LKB1* function in the mouse

LKB1 is evolutionarily conserved in animal organisms ranging from the Drosophila fruit fly and the C. Elegans nematode to primates, but is not found in yeast, plants or bacteria (Homologene record). The mouse homolog of *LKB1*, *Lkb1*, lies in a syngeneic locus on mouse chromosome 10 and is very highly conserved, giving rise to a protein with 90% identical and 93% similar amino acids as in human LKB1 (Figure 2). The high interspecies similarity has facilitated the study of Peutz-Jeghers syndrome and LKB1 functions using animal models, especially mouse models.

As would be expected for such a conserved gene, several mouse models have shown LKB1 function to be necessary for the survival of the organism. Systemic homozygous inactivation of *Lkb1* has been shown lethal both during embryogenesis, mainly as the vascular system fails to form correctly in the *Lkb1* knockout embryos (Ylikorkala et al. 2001; Jishage et al. 2002), and when induced in adult mice, as the hematopoietic stem cells undergo a process of rapid proliferation and subsequent cell death, resulting in loss of all blood cell types (Gan et al. 2010; Gurumurthy et al. 2010; Nakada et al. 2010) (Table 1).

6.1 Heterozygous Lkb1 knockouts

The *Lkb1*$^{+/-}$ mouse functions as a model of Peutz-Jeghers Syndrome, and gene knockout strategies that cause this genotype have been published by altogether five different research groups (Bardeesy et al. 2002; Jishage et al. 2002; Miyoshi et al. 2002; Rossi et al. 2002) (Wei et al. 2005) (Table 2) and phenotypes observed in at least 4 mouse background strains FVB (Contreras et al. 2008) and FVB/N (Shackelford et al. 2009), C57Bl/6J (Wei et al. 2005; Robinson et al. 2008), 129/sv (Robinson et al. 2008)), and CD1 (Rossi et al. 2002).

Especially for PJS gastrointestinal polyposis, the *Lkb1*$^{+/-}$ mouse is a very good model, as polyps develop in 100% of animals. It has served to first identify the upregulation of cyclooxygenase-2 (COX-2) as a feature of PJS polyposis (Rossi et al. 2002), a phenomenon later supported by studies from several different groups (De Leng et al. 2003; McGarrity et al. 2003; Wei et al. 2003; Takeda et al. 2004). The *Lkb1*$^{+/-}$ mice also display some spontaneous cancer development, in liver (Nakau et al. 2002) and endometrium (Contreras et al. 2008), but no pigmentation defects corresponding to PJS lentigines have been reported.

A hypomorph *Lkb1*^{fl} – allele, interestingly, does not cause PJS polyp formation, neither in heterozygote *Lkb1*$^{+/fl}$ (Sakamoto et al. 2005) nor in the homozygote *Lkb1*$^{fl/fl}$ (Sakamoto et al. 2005; Huang et al. 2008)(Table 2) mice, although also in these mice, the expression of Lkb1 is reduced to its half or below. Upon further reduction of the LKB1 expression, however, in simultaneously *AhCRe* transgenic mice, treated with beta-naphtoflavone, polyposis has been induced also in this model, although with a longer delay than in other models (Shorning et al. 2012).

6.2 Epithelial Lkb1 knockouts and cancer

More evidently than in the *Lkb1*$^{+/-}$ model, several mouse models of homozygous *Lkb1* loss present with cancer, when *Lkb1* is lost in epithelial tissues such as skin, prostate, mammary gland or endometrium (Gurumurthy et al. 2008; Pearson et al. 2008; McCarthy et al. 2009; Contreras et al. 2010) (Table 1). This potential for malignant transformation in homozygous *Lkb1* knockout models goes well with the observation that the wildtype *Lkb1* allele is retained in mouse polyps (Miyoshi et al. 2002; Rossi et al. 2002) (no loss of heterozygosity, LOH) and in a subset of PJS patient polyps (Entius et al. 2001; Miyaki et al. 2000; De Leng et al. 2003; Wang et al. 1999), but not in PJS cancers, where there is either LOH of *LKB1* (Wang et al. 1999; Entius et al. 2001; Sato et al. 2001; Kim et al. 2004; Nakanishi et al. 2004), or CpG island methylation of the *LKB1* promoter (Esteller

et al. 2001) in accordance with the Knudsen two-hit model for tumor suppression(Knudson 1971).

On the other hand, also heterozygous deletion of *Lkb1* has been shown sufficient to induce progression in several multifactor tumor models, like acceleration of lymphomas in *Pten*$^{+/-}$ mice (Huang et al. 2008), a completely new tumor spectrum in *p53*$^{-/-}$ mice (Wei et al. 2005; Ji et al. 2007); metastasizing lung adenocarcinomas, as well as progression of PanIN-lesions into pancreatic ductal carcinomas in *Kras*^{G12D} mice (Wei et al. 2005; Ji et al. 2007) (Table 3).

6.2 Stromal Lkb1 knockout and cancer

A very recent study (Tanwar et al. 2012) showed induction of endometrial cancer upon conditional homozygous ablation of Lkb1 in the stromal cells of the female reproductive tract using *Muellerian inhibiting substance receptor 2* (*Misr2*)-*Cre*. In this model, endometrial adenocarcinoma formation did not require any epithelial *Lkb1* mutation. Carcinoma formation was, however, greatly enhanced with simultaneous heterozygous deletion of *Lkb1* in the epithelial compartment underlining the importance of crosstalk between the two compartments.

Table 1. Embryonic stage homozygous (hmz) Lkb1 knockouts

Genotype	Tissue involved	Phenotype	References
Lkb1$^{-/-}$	all tissues	lethality around E8.5-9.5, neural tube defects, mesenchymal cell death, vascular and placental abnormalities, MEF:s produce increased VEGF, are resistant to senescence under hypoxia and to transformation by Ha-Ras	(Ylikorkala et al. 2001; Bardeesy et al. 2002; Jishage et al. 2002)
Lkb1$^{lox/lox}$*;Mox2-Cre*	all epiblast derived	No rescue of the *Lkb1*$^{-/-}$ phenotype	(Londesborough et al. 2008)
Lkb1$^{lox/-}$*;Tie1-Cre*	hmz in endothelium het in other tissues,	Survival until E11.5, decreased vascularization and hemorrhages, recruitment of vascular smooth muscle cells impaired, TGF-beta signaling implicated	(Londesborough et al. 2008)
Lkb1$^{lox/lox}$*;Tie2-Cre*	hmz in endothelium, hematopoietic cells	embryonic lethality	(Ohashi et al. 2010)
Lkb1$^{fl/fl}$	hmz Lkb1S knockout all tissues total Lkb1 5-10fold down (hypomorph) due to loss of Lkb1S	30% embryonic lethal, males infertile as junctions (ectoplasmic specializations) between sertoli cells and spermatids are not dissolved and spermatids brake	(Sakamoto et al. 2005; Towler et al. 2008) (Denison et al. 2010)
Lkb1$^{fl/fl}$*;Mck-Cre*	hmz in skeletal and cardiac muscle, others hypomorph	Enlarged atria, smaller ventricles, cardiomyocyte arrangement normal, reduced phosphorylation of AMPKα2 and ACC, AMPKα1 unaffected in cardiomyocytes.	(Sakamoto et al. 2006; Habets et al. 2009)
		Less AMPK activation, less glucose uptake in skeletal muscle, contraction strength and hypertrophic response to prolonged contractions normal	(Sakamoto et al. 2005; McGee et al. 2008)
Lkb1$^{lox/lox}$*;Mck-Cre*	skeletal and cardiac muscle	Reduced weight-bearing muscle mass, reduced voluntary running, earlier fatigue and poor recovery. mitochondrial proteins downregulated in different skeletal muscles, also PGC-1 down, more type IIb and less type IIx fibres, increased glucose tolerance by increased muscle glucose uptake.	(Thomson et al. 2007a, 2007b) (Koh et al. 2006) (Brown et al. 2011; Smith et al. 2011)
		In females, progressive myopathy in hindlimbs,with similar features decraesed type II A/D-fibre and increased type IIB-fibre content, greater fatigue and slower relaxation, mitochondrial content and thickness of subsarcolemmal layer reduced.	(Thomson et al. 2010)

Genotype	Tissue involved	Phenotype	References
Lkb1$^{lox/lox}$*;Mck-Cre*	skeletal and cardiac muscle	Heart failure, common in females, heart weight 10% higher, but protein concentration 10% lower, increased left ventricular diameter, 10% decreased heart rate, cardiac output 40% decreased, 70% decreased upon ischaemia+reperfusion, mTOR pathway implicated	(Thomson et al. 2007; Thomson et al. 2010) (Jessen et al. 2010)
Lkb1$^{lox/lox}$*;αMHC-Cre*	cardiac muscle hmz	biatrial enlargement, left ventricular hypertrophy, systolic dysfunction, atrial fibrillation phenotype similar to dn-CREB-TG mice	(Ikeda et al. 2009)
Lkb1$^{fl/fl}$*;POMC-Cre*	POMC neurons hmz others hypomorph	POMC-cells developed normally, reduced alpha-MSH secretion and reduced central melanocortin tone, females displayed loss of insulin suppression of gluconeogenesis in liver and reduced liver glycogen synthesis	(Claret et al. 2011)
Lkb1$^{fl/fl}$*;Albumin-Cre*	liver hmz others hypomorph	no functional bile canaliculi, polarity defect in ductal cells, lethal due to biliary stasis	(Woods et al. 2011)
Lkb1$^{lox/lox}$*;Emx-Cre*	neural progenitors in dorsal telencephalon	Thinner cortex, death of postmitotic neurons, agenesis of the corpus callosum, reduced cortical axons, SAD kinases (BRSK1/2) implicated in axon formation	(Barnes et al. 2007)
Lkb1-RNAi$^{-pCAG\text{-}IRES\text{-}GFP}$, in utero electroporation	ventricular zone, lower intermediate zone neurons	Increased distance between centrosome and nucleus, disrupted centrosome forward movement, multiple Tau-1-positive neurites, migration arrest within the IZ, cells reaching cortical plate had axon-like neurite in opposite direction to wt. Phenocopied by S9A-GSK3β or dominant negative APC electroporation.	(Asada et al. 2007; Asada and Sanada 2010)
Lkb1$^{lox/lox}$*;Rip2-Cre*	spinal cord, β-cells, some hypothalamic neurons	Spinal cord degeneration from 7-8 weeks, primarily in ascending tracts, with axon demyelinization, disorganized neurofilaments and microtubules	(Sun et al. 2010a)
		Increased β-cell and islet size, aberrant rosettes, enhanced insulin response decrease in feeding with reduced expression of hypothalamic NPY and POMC	(Sun et al. 2010b)
Lkb1$^{lox/lox}$*;Pdx1-Cre*	all pancreas	all cell lineages develop, islets smaller and less compact , pancreatic failure death by 2-3mo , 100% develop benign mucinous cystadenomas,	(Hezel et al. 2008; Morton et al. 2010)

Genotype	Tissue involved	Phenotype	References
Lkb1$^{lox/lox}$;K14-Cre	epidermal tissues	delayed hair growth, narrower hair shafts, increased erythema, follicular plugging, corneal hyperkeratinization, squamocellular carcinoma in 15%	(Gurumurthy et al. 2008)
Lkb1$^{fl/fl}$;Ah-Cre	prostate hmz other tissues hypomorph	100% atypical hyperplasia and bulbourethral gland cysts, 83% prostate intraepithelial neoplasia, 39% hyperplasia of the urethra, 11% seminal vesicle squamous metaplasia, lethality by 200 days. More nuclear β-catenin, p-Gsk3β, and Wnt signaling, reduction in Pten activation	(Pearson et al. 2008)
Lkb1$^{lox/lox}$;Sprrf2-Cre	muellerian epithelium, urothelium	invasive endometrial cancer in 100%, leading to death by local invasion, one case of systemic metastases	(Contreras et al. 2010)
Lkb1$^{lox/lox}$;Misr2-Cre	muellerian stromal cells	Oviductal cysts by 18 weeks, endometrial epithelial hyperplasia and endometrial carcinoma by 24 weeks. Activation of mTOR signaling in the carcinomas.	(Tanwar et al. 2012)
Lkb1$^{lox/-}$;Misr2-Cre	muellerian stromal cells all other tissues het	Phenotype same as in *Lkb1$^{lox/lox}$;Misr2-Cre,* but the endometrial tumors are bigger, more aggressive and invasive	(Tanwar et al. 2012)

Table 1. Homozygous Lkb1 knockouts in adult tissue (inducible knockouts)

Genotype	Tissue involved	Phenotype	References
Lkb1$^{lox/lox}$*;Rosa26-CreERT2* + tamoxifen i.p.	all tissues	Severe pancytopenia within 1 week, lethality within 30 days, first days after ablation hematopoietic stem cells (HSC) proliferate, then die through apoptosis, wt mice reconstituted with Lkb1 KO bonemarrow die within 60 days	(Gan et al. 2010; Gurumurthy et al. 2010)
Lkb1$^{lox/lox}$*;Ubc-CreERT2* + tamoxifen i.p.	all tissues	HSC phenotype as *Lkb1*$^{lox/lox}$*;Mx1-Cre* below	(Nakada et al. 2010)
Lkb1$^{lox/lox}$*;Mx1-Cre* +pIpC induction	variable knockout effect, in most tissues	Pancytopenia in 24-35 days, bonemarrow fails to reconstitute wt mice, lethality within 120 days due to pancytopenia, HSC proliferate more, but die, become aneuploid with supernumerary centrosomes and aberrant mitotic spindles.	(Gurumurthy et al. 2010; Nakada et al. 2010)
Lkb1$^{lox/lox}$*;Pdx1-CreERT2* + tamoxifen i.p.	pancreas	Larger β-cells, more insulin in islets, larger insulin output, higher proliferation, rozettes lost polarity, nuclei and primary cilia displaced, polarity defect partially mimicked by Par1b knockout, hypertrophy reversed by mTOR inactivation	(Fu et al. 2009; Granot et al. 2009)
Lkb1$^{lox/lox}$ + i.v. infection With Ad-CRE or CMV-Cre	liver	fasting and fed state hyperglycaemia, increased gluconeogenesis due to active PGC1α +higher transcription of gluconeogenetic enzymes, adiponectin reverses partially	(Shaw et al. 2005; Miller et al. 2011)
Lkb1$^{lox/lox}$*;BLG-Cre* +2 rounds of pregnancy	mammary gland	GII invasive ductal carcinomas or solid papillary carcinomas within 46-85 weeks, histological features similar to PJS breast cancers, luminal type transcriptome.	(McCarthy et al. 2009)
Lkb1$^{lox/lox;}$*TagIn*$^{(SM\text{-}CreERT2(ki)}$ + tamoxifen i.p.	mesenchymal derived smooth muscle cells	PJS gastrointestinal polyposis, by 11 months, as *Lkb1*$^{lox/+;}$*TagIn*$^{(SM\text{-}CreERT2(ki)}$ mice.	(Katajisto et al. 2008)
Lkb1$^{fl/fl}$*;Ah-Cre* +i.p. β-naphthoflavone	many tissues hmz, at least prostate, liver, gastro-intestinal epithelium, others hypomorph	PJS gastrointestinal polyposis around 270 days after induction, GI epithelium shows appearance of intermediate cells and increase in angiotensin related signaling molecules, especially Ang II and renin, also defective Par-1 signaling implicated	(Shorning et al. 2009; Shorning et al. 2011; Shorning et al. 2012)
Lkb1$^{lox/lox}$ + AdCre intranasally	lung	No tumor phenotype observed	(Ji et al. 2007)

Table 2. Heterozygous (het) and other incomplete knockouts of Lkb1

Genotype	Tissue involved	Phenotype	References
Lkb1$^{+/-}$	all tissues het	PJS gastrointestinal polyposis, polyp COX-2 upregulated, Wnt-signaling deregulated, polyp mTOR signaling upregulated Osteoblastosis and benign osteogenic tumors leading to paralysis well-differentiated endometrial adenocarcinomas in 50% of females Hepatocellular carcinomas in 70% of males	(Bardeesy et al. 2002; Jishage et al. 2002; Miyoshi et al. 2002) (Rossi et al. 2002; Takeda et al. 2004) (Lai et al. 2011), (Shaw et al. 2004b) (Robinson et al. 2008) (Contreras et al. 2008) (Nakau et al. 2002)
Lkb1$^{fl/fl}$	all tissues hypomorph 5-10 fold downregulation	30% embryonic lethal, no polyps, males infertile Reduced activity of AMPKα1, SIK2 and SIK3 in white adipose (see also complete knockouts)	(Sakamoto et al. 2005; Huang et al. 2008) (Gormand et al. 2011)
Lkb1$^{lox/+;}$*Mx1-Cre* induced with by pIpC by P15	most tissues het, knockout effect variable	No observed phenotype after 1-2mo	(Gurumurthy et al. 2010)
Lkb1$^{fl/+}$	all tissues ≤50% down Lkb1S het	males fertile, sperm counts normal	(Denison et al. 2010)
Lkb1$^{lox/+;}$*TagIn*$^{(SM\text{-}CreERT2(ki)}$ + i.p. tamoxifen	mesenchymal derived smooth muscle het	PJS gastrointestinal polyposis, polyp mTOR signaling upregulated	(Katajisto et al. 2008)
Lkb1$^{lox/+;}$*Lck-Cre*	maturing thymocytes het	15% less thymocytes than wildtype	(Cao et al. 2010)
Lkb1lox/+;Tie2Tg/+	endothelial cells het, hematopoietic cells het	impaired revascularization after ischaemia and impaired networking of endothelial cells	(Ohashi et al. 2010)
Lkb1$^{lox/+;}$*Mck-Cre*	skeletal and cardiac muscle het	25% reduction in Lkb1 expression in muscle, but phenotype normal	(Jessen et al. 2010)
Lkb1$^{lox/+;}$*Sprrf2-Cre*	muellerian epithelium het urothelium het	one case of benign uterine tumor	(Contreras et al. 2010)
Lkb1$^{lox/+;}$*Pdx1-Cre*	all pancreas het (by E8.5)	pancreas normal, disease free for 1.5 years	(Hezel et al. 2008; Morton et al. 2010)

Table 3. Incomplete Lkb1 knockouts combined with other tumorigenic manipulations

Genotype	Tissue involved	Phenotype	References
Lkb1$^{+/-}$;*p53*$^{+/-}$	all tissues Lkb1 het	Reduced survival, accelerated polyposis	(Wei et al. 2005; Takeda et al. 2006)
Lkb1$^{+/-}$;*p53*$^{-/-}$	all tissues Lkb1 het	Reduced survival, accelerated polyposis, new tumor spectrum: bladder, small intestinal, lung, mammary adenocarcinomas, stromal ovarian tumors, all tumors rare. LOH of Lkb1 in intestinal tumor	(Wei et al. 2005; Takeda et al. 2006)
Lkb1$^{fl/+}$;*Pten*$^{+/-}$	all tissues ≤50% down (Lkb1)	Pten-type polyps and lymphomas accelerated	(Huang et al. 2008)
Lkb1$^{fl/fl}$;*Pten*$^{+/-}$	all tissues Lkb1 hypomorph 5-10 fold downregulation	50% embryonic lethality, Pten-type polyps and lymphomas accelerated	(Huang et al. 2008)
Lkb1$^{lox/+}$; *Pten*$^{lox/lox}$ *Misr2-Cre*	muellerian duct stroma	no phenotype	(Tanwar et al. 2012)
Lkb1$^{+/-}$;*Catnb*$^{lox/+}$ + i.v. AdCMV-Cre	all tissues Lkb1 het β-Catenin het in liver	More frequent and more multifocal hepatocellular carcinomas, histologically similar to *Lkb1*$^{+/-}$ tumors	(Miyoshi et al. 2009)
Lkb1$^{+/-}$ + DMBA treatment	all tissues Lkb1 het	Invasive skin and lung cancers in about 50% of mice, LOH of Lkb1 in skin cancers, Ras-pathway deregulation	(Gurumurthy et al. 2008)
Lkb1$^{+/-}$; $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intrnasally	all tissues Lkb1 het Kras active in lung	Lung adenocarcinomas, with 30% metastasizing, no LOH of Lkb1	(Ji et al. 2007)
Lkb1$^{+/lox}$; $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intranasally	lung Lkb1 het and Kras active	Lung adenocarcinomas, with 30% metastasizing, no LOH of Lkb1	(Ji et al. 2007)

Genotype	Tissue involved	Phenotype	References
Lkb1$^{+/lox}$*;Pdx1-Cre;* *lox-stop-lox**Kras*^{G12D} + AdCre intranasally	Pancreas Lkb1 het and Kras active	Increase in Kras-induced PanIn:s, higher grades, more proliferation, also pancreatic ductal adenocarcinomas, no LOH of Lkb1, p53 pathway downregulated	(Morton et al. 2010)
Lkb1$^{+/lox}$*;Pdx1-Cre;* *lox-stop-lox**Kras*^{G12D}*;p53*$^{R172H/+}$ + AdCre intranasally	Pancreas Lkb1 het, Kras active, also p53 het	phenotype same as *Lkb1*$^{+/lox}$*;Pdx1-Cre;* *lox-stop-lox**Kras*^{G12D}	(Morton et al. 2010)
Lkb1$^{+/lox}$*;Pdx1-Cre;* *lox-stop-lox**Kras*^{G12D}*;p21*$^{+/-}$ + AdCre intranasally	Pancreas Lkb1 het, Kras active, also p21 het	Kras phenotype same as *Lkb1*$^{+/lox}$*;Pdx1-Cre;* *lox-stop-lox**Kras*^{G12D}	(Morton et al. 2010)

Table 3. Homozygous Lkb1 knockouts combined with other tumorigenic manipulations

Genotype	Tissue involved	Phenotype	References
Lkb1$^{fl/fl}$;Pten$^{lox/lox}$;Ah-Cre +i.p. β-naphthoflavone	many tissues both hmz others Lkb1 hypomorph	Diffuse bladder epithelium hyperplasia with papillary growths, squamous metaplasia and nuclear pleiomorphism, no invasion, mTOR pathway and EMT markers upregulated	(Shorning et al. 2011)
Lkb1$^{lox/lox}$;K14-Cre + DMBA treatment	all tissues Lkb1 het	squamocellular carcinomas as in DMBA-treated *Lkb1$^{+/-}$* mice but with 100% prevalence	(Gurumurthy et al. 2008)
Lkb1$^{lox/lox}$; $^{lox\text{-}stop\text{-}lox}$Kras^{G12D} + AdCre intranasally	lung hmz, Kras active	Lung tumors with squamous morphology, 60% metastases, upregulation of EMT associated genes, Src-family kinases , FAK pathway activation	(Ji et al. 2007; Carretero et al. 2010)
Lkb1$^{lox/-}$; $^{lox\text{-}stop\text{-}lox}$Kras^{G12D} + AdCre intranasally	lung hmz, Kras active other tissues Lkb1 het	Tumor phenotype similar to *Lkb1$^{lox/lox}$; $^{lox\text{-}stop\text{-}lox}$Kras^{G12D}*	(Ji et al. 2007)
Lkb1$^{lox/lox}$; Pten$^{lox/lox}$ Misr2-Cre	muellerian duct stromal cells	endometrial carcinomas accelerated (found by 9weeks), oviductal cysts accelerated (found by 12 weeks), vaginal and cervical squamous cell tumors add to tumor spectrum	(Tanwar et al. 2012)

7. Treatments and treatment trials in Peutz-Jeghers syndrome and its mouse models

7.1 Treatments in clinical use

The currently used treatment of PJS is surgical, with extensive follow-up to detect tumors, and removal upon detection of both polyps and cancers according to standard guideline protocols for cancer surgery. To illustrate the substantial scope of this regimen, the follow-up protocol recommended by the USA National Comprehensive Cancer Network (as of 2nd November 2009, http://www.nccn.org/) is listed in Table 4.

Table 4. Recommended follow-up for Peutz-Jeghers patients.

Examination	Starting age	Frequency	Sex
Stomach and small intestine endoscopy	10	every 2-3 years	both
CT enterography/small intestine enteroclysis	10	every 2-3 years	both
colonoscopy	late teens	every 2-3 years	both
testicular exam, observation for femininization	10	annually	males
clinical breast exam	25	every 6 months	females
pelvic exam and pap-smear (+ultrasound exam)	25	annually	females
pancreas screening by magnetic resonance cholangiopancreatography and/or ultrasound	30	every 1-2 years	both
CA 19-9 blood test	30	every 1-2 years	both
+ preventive measures against lung cancer as for the general population			both

7.2 Experimental treatments

In *Lkb1*$^{+/-}$ mice, the Cyclooxygenase (COX)-2 inhibitor celecoxib suppresses polyposis (II), and rapamycin, an mTOR inhibitor, has been found to reduce both polyposis (Wei et al. 2008; Robinson et al. 2009; Shackelford et al. 2009; Wei et al. 2009) and endometrial cancers (Contreras et al. 2010) (Table 5).

While the molecular basis for assuming mTOR regulation plays a role in polyp formation and progression is clear (see e.g. Figure 3), pinpointing a molecular context for COX-2 in PJS polyposis is harder. The cyclooxygenases (1 and 2) function as rate-limiting enzymes in the prostaglandin synthesis, and COX-2 can be induced by various stimuli,

e.g. during inflammation. LKB1 signaling has been shown to directly suppress the transcription of COX-2 in lung cancer (Upadhyay et al. 2006; Ratovitski et al. 2010), but COX-2 could also be indirectly induced (Pham et al. 2008) in the PJS polyps by a dysregulated local renin-angiotensin signaling system (Shorning et al. 2012).

Also in PJS patients, two different experimental medical treatments have been reported, of which one study (II) included in this thesis used celecoxib against polyposis (II), and the other study used an mTOR inhibitor, everolimus, against polyposis and pancreatic cancer (Klumpen et al. 2011). Whereas COX-2 inhibitors are currently not recommended for use in PJS, due to their cardiovascular side effects, everolimus is now being evaluated in at least one larger clinical trial, providing some positive prospect for the development of a future treatment to the PJS patients.

Table 5. Treatment trials in Lkb1 mouse models

Genotype	Treatment	Outcome	References
Lkb1$^{+/-}$	Celecoxib COX-2 inhibitor	Polyposis attenuated, polyp vascularity (MVD) reduced	(II, Udd et al. 2004)
Lkb1$^{+/-}$ mTOR-inhibitor	Rapamycin	Polyposis attenuated, more efficiently in males, (MVD) reduced no effect on hepatocellular carcinoma incidence	(Wei et al. 2008; Wei et al. 2009; Robinson et al. 2009; Shackelford et al. 2009)
Lkb1$^{lox/lox}$*;Rosa26-CreERT2* + tamoxifen i.p.	rapamycin	No response	(Gan et al. 2010; Gurumurthy et al. 2010)
Lkb1$^{lox/lox}$*;Rosa26-CreERT2* + tamoxifen i.p.	metformin AMPK activator	No response	(Gan et al. 2010)
Lkb1$^{lox/lox}$*;Ubc-CreERT2* + tamoxifen i.p.	rapamycin	No response	(Nakada et al. 2010)
Lkb1$^{lox/lox}$*;Mx1-Cre* +pIpC induction	rapamycin	No response	(Gurumurthy et al. 2010; Nakada et al. 2010)
Lkb1$^{lox/lox}$*;Pdx1-CreERT2* + tamoxifen i.p.	rapamycin	β-cell hypertrophy reversed, polarity defect not affected	(Fu et al. 2009; Granot et al. 2009)
Lkb1$^{lox/lox}$*;Sprrf2-Cre*	rapamycin	endometrial cancers regressed to 50%, mTOR signaling normalized, tumors regrew after cessation of treatment	(Contreras et al. 2010)
Lkb1$^{lox/lox}$*;Misr2-Cre*	rapamycin	suppression of endometrial carcinogenesis, and of oviductal cyst formation	(Tanwar et al. 2012)
Lkb1$^{fl/fl}$*;Ah-Cre* +i.p. β-naphthoflavone	rapamycin	No effect on gastrointestinal epithelial phenotype	(Shorning et al. 2009)
Lkb1$^{lox/lox}$*;αMHC-Cre*	rapamycin	hypertrophy of cardiomyocytes reversed	(Ikeda et al. 2009)
Lkb1$^{lox/lox}$*;* $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intranasally	sunitinib RTK inhibitor	survival prolonged from 9.1 to 11.7 weeks, similar incidence of metastases	(Gandhi et al. 2009)

Genotype	Treatment	Outcome	References
Lkb1$^{lox/lox}$; $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intranasally	BAPN, LOX inhibitor)	reduced tumor burden, fewer large tumors and less invasion-promoting collagen deposits	(Gao et al. 2010)
Lkb1$^{lox/lox}$; $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intranasally	dasatinib, SRC inhibitor	No response	(Carretero et al. 2010)
Lkb1$^{lox/lox}$; $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intranasally	BEZ235, PI3K inhibitor + selumetinib, MEK inhibitor	No response	(Carretero et al. 2010)
Lkb1$^{lox/lox}$; $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intranasally	dasatinib + BEZ235+ selumetinib	Significant tumor regression	(Carretero et al. 2010)
Lkb1$^{lox/lox}$; $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intranasally	Docetaxel	No response	(Chen et al. 2010)
Lkb1$^{lox/lox}$; $^{lox\text{-}stop\text{-}lox}$*Kras*^{G12D} + AdCre intranasally	Docetaxel + selumetinib	No response	(Chen et al. 2010)
Lkb1$^{fl/+}$;*Pten*$^{+/-}$	AZD8055 mTOR inhibitor	lymphomas regressed to 50%, regrew after cessation	(Garcia-Martinez et al. 2011)
Lkb1$^{fl/+}$;*Pten*$^{+/-}$	GDC-0941 PI3K inhibitor	lymphomas regressed to 50%, regrew after cessation, 40% regression by second treatment, second cessation regrowth speed same as first	(Garcia-Martinez et al. 2011) (Wullschleger et al. 2012)
Lkb1$^{fl/+}$;*Pten*$^{+/-}$	metformin AMPK activator	lymphomas marginally delayed, 100% affected by 1 year	(Huang et al. 2008)
Lkb1$^{fl/+}$;*Pten*$^{+/-}$	phenformin AMPK activator	lymphomas delayed, 20% disease free by 1 year	(Huang et al. 2008)
Lkb1$^{fl/+}$;*Pten*$^{+/-}$	A-769662 AMPK activator	lymphomas delayed, 10% disease free by 1 year	(Huang et al. 2008)
Lkb1$^{fl/fl}$;*Pten*$^{lox/lox}$;*Ah-Cre* +i.p. β-naphthoflavone	rapamycin	Bladder hyperplasia reduced, reduced proliferation, mTOR and EMT markers normalized	(Shorning et al. 2011)

AIMS OF THE STUDY

The general aim of this thesis project was to gain further insight into the role of the LKB1 inactivation in pathogenesis of Peutz-Jeghers Syndrome, hoping both that the findings may benefit Peutz-Jeghers Syndrome patients, and that the new knowledge may help us to better understand more common cancers and diseases.

Detailed aims of the thesis subprojects:

I To facilitate the search for LKB1 substrates by establishing cell lines with and without LKB1 kinase activity, from primary cells

II To characterize the effect of heterozygous *LKB1* inactivation on the differentiation of gastric epithelium in the formation of Peutz-Jeghers syndrome polyposis

III To study the effects of COX-2 inhibition on Peutz-Jeghers polyposis, and to test whether COX-2 inhibitors could be used for polyp chemotherapy.

IV To model the effects of DNA damage in Peutz-Jeghers syndrome by carcinogen induction in the *Lkb1*$^{+/-}$ mouse, and to evaluate the effects of COX-2 inhibition in this setting

MATERIALS AND METHODS

1. Materials

Materials generated for and /or used in the thesis studies are listed below with references, and the according original publications are referred to in Roman numerals.

Mouse lines	**source/reference**	**used in**
CD1	Jackson Laboratories	I-IV
C57BL/6	Jackson Laboratories	II, III, IV
Lkb1	(Ylikorkala et al. 2001)	I-IV
Cox-2	(Dinchuk et al. 1995)	II

Cell lines	**source/reference**	**used in**
HeLa/wt LKB1, /kinase dead LKB1	(Sapkota et al. 2002)	I
Immortal wt and Lkb1$^{-/-}$ MEFs	by author, (Hawley et al. 2003)	I
HEK 293 cells	ATCC	I

Antibodies	**source/reference**	**used in**
anti-BRSK1, -BRSK2, -NUAK1, -NUAK2, -QIK, -SIK, -QSK, -MARK1, -MARK4, -MELK, -AMPKα1, and -AMPKα1,α2	(Lizcano et al. 2004)	I
anti-MARK3 and MARK2 (#05-680)	Upstate Biotech	I
anti-T-loop-phosphorylated AMPK	(Sugden et al. 1999)	I
anti-Lkb1	(Sapkota et al. 2001)	I
anti-LKB1	(Boudeau et al. 2003b)	I
anti-CD31 (#01950D)	PharMingen	II
anti-COX-2 (#160126)	Cayman Chemical	II
anti-actin (#AC-40)	Sigma-Aldrich	II
anti-pepsinogen C (Pg-1)	(Furihata et al. 1973)	III, IV
anti-intrinsic factor	(Howard et al. 1996)	III
anti-TFF2 (hSP)	(Elia et al. 1994)	III
anti-H^+/K^+-ATPase (HA3-923)	Affinity Bioreagent	III
anti-Cdx2 (MU392A-U9)	Bio-Genex Laboratories	III
anti-gastrin (#A0568), -lysozyme (#A0099) -chromogranin A(#A0430), -Ki-67 (TEC-3)	DakoCytomation	III
anti-desmin (DE-R-11),-Muc6 (NCLMUC-6)	Novocastra	III
anti-SMA (#A5691)	Sigma-Aldrich	III
Griffonia simplicifolia lectin II (B-1215)	Vector	III
anti- HA epitope	Roche	I
anti-GST and -FLAG epitopes	Sigma-Aldrich	I
Anti-*myc* antibodies	(Lizcano et al. 2004)	I
horseradish peroxidase secondary	Pierce	I
biotinylated secondary antibodies	Vector	II-IV
FITC anti-mouse IgM	Vector	III

AlexaFluor488 secondary antibody	Invitrogen	III
Antibodies	**source/reference**	**used in**
ARK-kit for antibody biotinylation	DakoCytomation	III
ApopTag apoptosis detection kit	Chemicon/Serologicals	II
Tyramide Signal Amplification-kit	Perkin Elmer	II

Gene epression arrays	**source/reference**	**used in**
Mg-U74a2 GeneChip	Affymetrix	III
RNEasy-kit	Qiagen	III
Superscript II kit	Qiagen	III
ENZO kit	Enzo Life Sciences	III

DNA Cloning Reagents	**source/reference**	**used in**
Quikchange mutagenesis kit	Stratagene	I
Mega kit	Qiagen	I
DYEnamic ET terminator chemistry	Amersham Biosciences	I
pCR2.1-TOPO vector	Invitrogen	I
pEBG2T vector	(Sanchez et al. 1994)	I
pGEX6P-1 vector	Amersham	I

Kinase Assay Reagents	**source/reference**	**used in**
P81 phosphocellulose paper	Whatman	I
P32ATP	Amersham biosciences	I
AMARA peptide	(Dale et al. 1995)	I
LKBtide peptide	(Lizcano et al. 2004)	I
Vydac 218TP5215 C18 columns	Separations Group	I
PHOS-select Iron chelate gel	Sigma-Aldrich	I

Other Major Reagents	**source/reference**	**used in**
Phenformin	Sigma-Aldrich	I
AICA-riboside	Toronto Res. Chemicals, Inc.	I
Celecoxib	Searle-Pharmacia, Pfizer	II, IV
N-methyl-N-nitrosourea	Sigma-Aldrich	IV

2. Methods

All methods used in the thesis are described in detail in the four original publications, and are listed alphabetically below with references to the respective original publication (Roman numerals). The methods used by the author are described below the list in further detail.

Method/Protocol	**used in**
Affymetrix expression profiling	III
Antibody raising and affinity purification	I
Tissue/cell culture	I
DNA fragment cloning	I
Fluorescence microscopy	III
Gastroscopy	II
Generation of immortalized MEFs	I

Genotyping by PCR	I-IV
Immunohistochemistry	II-IV
Method/Protocol	**used in**
In vitro kinase assay	I
Isolation of primary MEFs	I
Light microscopy	II-IV
Mouse breeding/crossing	I-IV
Phosphopeptide sequence analysis	I
Protein overexpression	I
RNA extraction	III
Sequencing	I
Site-directed mutagenesis	I
Tissue preparation for histology	II-IV
Tryptic phosphorylation mapping	I
TUNEL assay	II
Western blot analysis	I, II

Mouse maintenance (I-IV)

All mice had free access to food and water before and throughout the studies. Animal health was monitored according to the recommendations of the animal welfare committee of the University of Helsinki, and ethical permissions for the studies were obtained from the State Provincial Office of Southern Finland. The given permit numbers were STU48A and STU536A for *Lkb1*-knockout mouse breeding (I-IV), STU 413A for the celecoxib study (II) as well as STU1222A and STU444A for the MNU and celecoxib study (IV). Celecoxib powder was mixed at a concentration of 1500 parts per million (II) or 150 parts per million (IV) with global rodent chow (Harlan Teklad), and stored at -20°C until being fed to the mice. N-methyl-N-nitrosourea was diluted freshly into the drinking water of the mice, at a concentration of 120ppm and bottles replaced every 48-72h (IV). Upon weaning of the experimental animals, tail-end or ear-clipping samples were incubated with Proteinase K, before samples were genotyped with primers designed against the wildtype or knockout allele of *Lkb1* (Ylikorkala et al. 2001) (I-IV) or of *COX-2* (Dinchuk et al. 1995) (II). Genotyping of all animals was also performed (I), or repeated (II-IV) upon dissection.

Mouse embryo fibroblast (MEF) culture (I)

Wildtype and $Lkb^{-/-}$ E9.5 embryos were mechanically fragmented and outgrowing cells were cultured for 5 days, in Dulbecco's modified Eagle's Medium supplemented with penicillin, streptomycin, glutamine, 10% fetal bovine serum, and 10% conditioned medium from wild-type MEFs. After this initial establishment of the cultures, the cells were passaged according to a modified 3T3 protocol (Denhardt et al. 1991).

Tissue analyses (II-IV)

For histological analysis, mouse tissue samples were dissected and fixed overnight in 2-4% paraformaldehyde, followed by microdissection and further sample selection under an inverted microscope. Samples were then embedded in paraffin and sectioned using standard techniques. All immunochemistry was performed on deparaffinized and rehydrated sections. When appropriate, endogenous peroxidase was quenched in 3%

hydrogen peroxide. Epitope retrieval was achieved either by heat induction with DAKO Target Retrieval (DakoCytomation), or by peptic induction with trypsin (Difco Laboratories). Some antibodies were used without epitope retrieving pretreatment. Immunostainings were counterstained with hematoxylin (Thermo Fisher Scientific) or eosin (II-IV).

Microvessel density (MVD) was scored manually during microscopy from sections stained for CD31, as described (Seno et al. 2002)(II). Pepsinogen altered pyloric glands (PAPG) were also scored manually during microscopy as the ratio of glands negative for pepsinogen C staining (III). The mean size of TFF2 expressing cells was scored as the cross section area in micrographs of immunostained tissue sections, using ImageJ software (*http://rsb.info.nih.gov/ij/*) (III). Numbers of different cell types per gastric unit were scored manually from micrographs of histological sections (III).

For immunoblot analysis, snap frozen tissue was lysed in 1X Laemmli buffer with Qbiogene Lysing Matrix D in a FastPrep homogenizer (QBiogene, Carlsbad, CA). Western blotting analysis was performed using standard protocols.

Patient studies

Permissions for patient studies were obtained from the Finnish National Agency for Medicines (now Finnish Medicines Agency, Fimea) and from the ethical board of the department of surgery at the University of Helsinki Central Hospital (II).

Archived paraffin-embedded samples of polyps and biopsies of normal gastric tissue from Peutz-Jeghers patients were obtained from the Helsinki University Central Hospital Pathology Department (II, III).

Statistical analyses

Statistical analyses were performed using the SPSS statistical software package (Apache Software Foundation, SPSS Inc.) II, III, or the PASW statistics 18.0 package (SPSS Inc.) IV.

RESULTS AND DISCUSSION

1. LKB1 phosphorylates and activates kinases of the AMPK family

1.1 LKB1:STRAD:Mo25 phosphorylates and activates 13 AMPK-related kinases in vitro (I)

In a cell-free system, *in vitro*, BRSK1, BRSK2, NUAK1, NUAK2, SIK, QIK, QSK, MARK1, MARK2, MARK3 and MARK4 were shown to phosphorylate AMARA peptide, previously known to phosphorylate AMPK (Dale et al. 1995), and this phosphorylation was 50-200-fold increased when LKB1:STRAD:Mo25 complex was present, but not when LKB1 was in isolation. MELK also phosphorylated AMARA peptide, but this activity was not affected by the LKB1:STRAD:Mo25 complex. This in vitro data showed that phosphorylation by the LKB1 complex serves to activate the AMPK family kinases except MELK.

T-loop threonines (T) corresponding to the Thr172 in AMPKα1 known to be phosphorylated by the LKB1:STRAD:Mo25 complex (Hawley et al. 2003), were converted to alanines (A) and to glutamic acids (E). As expected for manipulations of an activating phosphorylation site, TA-mutations, preventing phosphorylation, abolished or decreased kinase activity, whereas most TE-mutants, partly mimicking phosphorylated threonine residues, behaved as constitutively activated kinases. Although TE-mutants of MARK1-3, did not show this phenotype, there is no reason to assume the physiological role of the site to be different in these kinases. To address the same issue by an independent technology, the localization of the LKB1 phosphorylation site on BRSK2 and NUAK2 was reconfirmed with analysis of ^{32}P-labelled tryptic digests. Taken together, these data demonstrated that a specific LKB1 phosphorylation site corresponding to the Thr172 in AMPKα1 was conserved on the T-loop of the AMPK family kinases.

1.2 LKB1 is required *in vivo* for activation of AMPK and several AMPK-related kinases (I)

Peptide antibodies against the AMPK-related kinases were generated, and their ability to immunoprecipitate (IP) the kinases was tested on lysates of HEK-293 cells, in which the respective kinases had been overexpressed. For MARK2/3 a commercial antibody was available. IP of NUAK2, QIK, QSK, SIK, MARK1, and MARK4 was performed from *Lkb1*$^{-/-}$, and *Lkb1*$^{+/+}$ mouse embryonic fibroblasts (MEFs), and a kinase activity assay performed. This experiment revealed a 7-35-fold decrease in the kinase activities of NUAK2, QIK, QSK, SIK, MARK1, and MARK4 and a ~3fold decrease in MARK2/3 kinase activity in the LKB1 knockout cells. There was no significant decrease in the expression of any of these kinases in the *Lkb1*$^{-/-}$ MEFs, as shown by immunoblotting.

This data indicated that, *in vivo*, in Lkb1 deficient cells, the activity of the LKB1 substrates was reduced, due to the absence of activating phosphorylation.

1.3 Phenformin does not activate LKB1 (I)

When treated with phenformin, *Lkb1*$^{-/-}$ MEFs showed no activation of AMPKα1, whereas *Lkb1*$^{+/+}$ MEFs showed an upregulation of AMPKα1 activity, as previously shown (Hawley et al. 2003). Interestingly, however, none of the activities of the other AMPK related kinases was affected in either cell type. This data showed, that although AMPK activation by phenformin is dependent on the presence of LKB1, phenformin does not cause AMPK activation by activating LKB1. These studies were repeated also in normal HeLa cells, which do not express LKB1 (Hawley et al. 2003), and in HeLa cells stably expressing wildtype or catalytically inactive LKB1 (Sapkota et al. 2002; Hawley et al. 2003) with similar results.

2. Cyclooxygenase-2 Promotes PJS polyposis

2.1 In *Lkb1*$^{+/-}$ mice, COX-2 collaborates with LKB1 in driving polyposis (II)

Cox-2 expression is found in *Lkb1*$^{+/-}$ mouse polyps (Rossi et al. 2002; Takeda et al. 2004) and in PJS patient polyps (Wei et al. 2003; McGarrity et al. 2003). We saw a 40-60% prevalence of COX-2 expression in the polyps, depending on the size of the polyp, and a ~50% reduced *Lkb1*$^{+/-}$ mouse gastric polyp burden in *Cox-2*$^{-/-}$ and *Cox-2*$^{+/-}$ backgrounds. The number of large polyps was reduced more significantly than the number of small polyps, suggesting that COX-2 accelerates the growth of polyps rather than affecting the initiating steps of polyp formation.

2.2 COX-2 inhibitor treatment reduces PJS-type polyposis (II, IV)

Lkb1$^{+/-}$ mice were treated with COX-2 inhibitor celecoxib at 1500ppm (I), or 150ppm (IV), and polyp burden decreased ~7-fold with the higher dose, and ~4-fold with the lower dose. Treated *Lkb1*$^{+/-}$ polyps had a lower microvessel density, also found to correlate with COX-2 inhibitor response in the *Apc*^{Delta716} model of familial adenomatous polyposis (Seno et al. 2002). There were no changes in the morphological appearance of the polyps upon treatment, nor any increase in epithelial cell apoptosis.

PJS patient gastric polyposis was assessed by gastroscopy before and after a 6-month treatment with 200 mg celecoxib bidaily, corresponding to half the dose used in the treatment of familial adenomatous polyposis patients. Digital recordings of the

gastroscopies were analyzed by 5 independent evaluators, who graded the severity of the polyposis. In two out of six examined patients, significant improvement of the polyposis grading score was seen with celecoxib treatment, suggesting beneficial effects of COX-2 inhibitor treatment in at least a subset of PJS patients.

3. Gastrointestinal epithelial differentiation defects in PJS

3.1 Gastric gland cell differentiation is impaired in Peutz-Jeghers polyps (III, IV)

Gastric polyps from *Lkb1*$^{+/-}$ mice and PJS patients were studied for epithelial differentiation markers, and found to display either weak or no Pepsinogen C antibody reactivity (suggesting loss of antral gland, mucopeptic and chief cells). Fundic polyps also displayed absence of parietal cells as based on H^+/K^+-ATPase antibody staining and cell morphology. The presence of enteroendocrine cells, on the other hand (as based on Chromogranin A stainings) was deemed unaltered. The RNA-expression profiles of *Lkb1*$^{+/-}$ polyps and normal mucosa supported the immunohistochemical findings, with downregulation of genes attributed to the expression profiles recorded for chief cells (Mills et al. 2003) and parietal cells (Mills et al. 2001).

The glands of the mouse and patient polyps were occupied by Muc-6-expressing precursor gland cells. These cells did not fit to the description of "antralizing" or spasmolytic peptide expressing metaplasia (Goldenring and Nomura 2006), as there was no increase in spasmolytic peptide expression, no increase in the size of the cells expressing it, and no colocalizing expression of intrinsic factor. Also antralization, defined as incorrect specification of fundic epithelium into antral epithelium was not evident, as gastrin expression was seen widely in antral polyps and antral *Lkb1*$^{+/-}$ mucosa, but not in fundic polyps, nor in fundic *Lkb1*$^{+/-}$ mucosa. Taken together, this data suggested a block in the terminal differentiation process of the glandular cells of the gastric Peutz-Jeghers polyps, rather than a metaplastic process of "de-differentiation".

Gastric epithelial differentiation was also studied in the mucosa of young *Lkb1*$^{+/-}$ mice, prior to the onset of polyposis. In this setting, we also found a statistical difference in the occurrence of glands lacking Pepsinogen C expression. Some of these glands also had an aberrant pattern of cell proliferation (as shown by staining the Ki-67 epitope), there was, however, no stromal aberrations attributed to polyp development detectable at this point, suggesting aberrant epithelial differentiation to be the earliest sign of polyp initiation.

3.2 Secretory cell differentiation is impaired in intestinal Peutz-Jeghers polyps (III)

Lysozyme staining was absent from the crypts of small intestinal *Lkb1*$^{+/-}$ and PJS patient polyps suggesting loss of Paneth cells. This was supported also by Cdx-2 staining nuclei of the crypt cells, as this intestinal differentiation regulator should be downregulated to allow for Paneth cell specification (Blache et al. 2004). Also among normal appearing Alcian Blue-staining goblet cells, there were a large number of cells of similar morphology that also stained with lysozyme antibody, suggesting the increased presence of less differentiated so-called "intermediate" cells (Udd and Makela 2011). These findings indicated that apart from gastric gland cells, also intestinal secretory cells show impaired differentation in PJS polyposis, and suggested the disruption of a common regulatory mechanism for the differentiation of the gastric gland cells and the intestinal secretory cells.

4. DNA damage accelerates *Lkb1*$^{+/-}$ polyposis (IV)

4.1 DNA damage potentiates *Lkb1*$^{+/-}$ polyp growth without malignant transformation (IV)

Lkb1$^{+/-}$ mice were treated with the DNA-alkylating agent N-Methylnitrosourea (MNU), causing decreased survival compared to wildtype mice undergoing the same treatment, and an almost 10-fold increase in gastric polyp burden compared to untreated *Lkb1*$^{+/-}$ mice. There was no change in polyp morphology upon treatment, and only one case of dysplasia was seen in a polyp. The size of the polyps was increased through enhanced epithelial proliferation. Polyp numbers, including the number of small polyps, were also dramatically increased, suggesting that additional mutations accelerate both the initiation and progression of PJS polyposis.

4.2 COX-2 inhibition does not prevent DNA-damage induced polyposis acceleration (IV)

Groups of *Lkb1*$^{+/-}$ and wildtype mice treated with MNU were subsequently also given celecoxib. This treatment decreased the mortality of the mice slightly, but not significantly. Also the gastric polyp burden was slightly reduced, but the reduction was not statistically significant. This suggested that treatment with COX-2 inhibitors might not be sufficient to prevent the progress of Peutz-Jeghers polyposis in the presence of additional DNA-damage.

4.3 Gastric epithelial differentiation defect unaffected by DNA damage

The occurrence of gastric glands lacking pepsinogen C expression is increased upon treatment of wildtype animals with carcinogens such as MNU (Yamamoto et al. 1997), and pepsinogen C expression can be normalized by COX-2 inhibitor treatment (Hu et al. 2004). In the *Lkb1*$^{+/-}$ mice, however, no further increase above the basal was observed upon MNU treatment, and no effect was seen with celecoxib, although the wildtype mice followed an expected pattern of increase and decrease. These observations suggest, that the mechanism underlying lack or loss of pepsinogen C expression is different in the *Lkb1*$^{+/-}$ setting, and rather a sign of nascent polyposis than of carcinogen exposure.

GENERAL DISCUSSION

Before our finding that the AMPK-related kinases are LKB1 substrates (I), the research fields of LKB1 biology, and of kinase biology were broadened by two major advances. The human kinome, the phylogenetic tree of all human kinases was published (Manning et al. 2002), and LKB1 itself was described to phosphorylate AMPK (Hawley et al. 2003). The sequence similarity of the kinases related to AMPK, then gave reason to study the whole family for interaction. Indeed, the link to AMPK itself was already very interesting, as it linked PJS to the other hamartoma syndromes (see Figure 4, in the Review of the Literature section) and surprisingly enough – to diabetes, in which AMPK regulation plays a central role (Viollet et al. 2009b).

Finding the other substrates diversified the view of the capacities of LKB1, fitting the optimistic nickname “Master Tumor Suppressor” branded by the author’s namesake (Yoo et al. 2002). Through its many substrates, LKB1 is positioned to regulate a multitude of cellular processes associated with polarity (asymmetric cell division, apicobasal determination of epithelial cells, neuronal axon specification, and directional migration of mesenchymal cells) (Jansen et al. 2009) intracellular transport(Asada and Sanada 2010), transcription (Katoh et al. 2006), and response to energy stress (van der Velden et al. 2011; Jeon et al. 2012).

The prominent role of LKB1 in the regulation of a wide variety of cell responses further reinforced interest in studying the molecular mechanisms of tumor formation in PJS. In addition, mutations in LKB1 have been found also in some of the most common cancer types, such as lung adenocarcinoma (Sanchez-Cespedes et al. 2002) and breast cancer (Shen et al. 2002), further highlighthing the central role of LKB1 in tumor biology in general.

The finding, that phenformin (an analogue of metformin, the well established drug for type II diabetes) did not function as an activator of LKB1 (I) diminished some of the enthusiasm for finding potential anti-cancer use for this kind of pharmacological agents, biguanides. A theoretical basis for antineoplastic function still remains however, with the confirmed link to AMPK and mTOR pathway regulation. Data from epidemiological studies have given variable results, some of which support a protective effect from long-term metformin treatment while others do not. Some anticancer effects of metformin have been demonstrated *in vitro*, and the subject remains a prime research topic (Pollak 2012).

Apart from the studying *LKB1*, and loss of LKB1 function on a cellular level, we also studied it on a tissue level, as we explored the differentiation of epithelial cells in the polyposis-prone gastrointestinal mucosa of *Lkb1*$^{+/-}$ mice and Peutz-Jeghers patients (III). We found that the differentiation into more specialized glandular cells was impaired in the stomach both in the pre-polypotic young mucosa, and more dramatically in the polyps. This finding was unexpected based on the previously accepted paradigm, that the Peutz-Jeghers epithelium is well differentiated, and contains all epithelial cell types and lineages native to the site of occurrence(Estrada and Spjut 1983; Tobi 1999). Since then, more evidence for pre-polypotic changes in epithelial stability due to LKB1 heterozygosity have been reported by Langeveld et al., who have shown that unaffected PJS patient intestinal epithelium has a more diverse pattern of genetic methylation than that of controls, and that the mucosal regeneration process in the crypts is protracted (Langeveld et al. 2012).

Although mesenchymal derived cells have been shown capable of driving Peutz-Jeghers polyp formation (Katajisto et al. 2008), these findings suggested a role also for the epithelial compartment in Peutz-Jeghers polyp formation. Either it could influence polyposis by providing an alternative pathway to polyp formation, as suggested by the findings of pre-polyposis foci of poor differentiation (III), aberrant methylation patterns and prolonged clonal cell expansion (Langeveld et al. 2012) in the epithelium prior to stromal aberrations. On the other hand, it could also function as a partner for epithelial-stromal interaction as suggested by the observation that polyposis is much more pronounced, upon concomitant loss of *Lkb1* in the epithelial compartment (Katajisto et al. 2008).

Reduced TGFβ signaling activity has been shown in the stromal smooth muscle lineage cells (Katajisto et al. 2008), and in Lkb1 deficient fibroblasts (Vaahtomeri et al. 2008), and may be involved in the signaling from stroma to epithelium, whereas the candidates for aberrant epithelial to stromal are less conspicuous. Perhaps this route of signaling is influenced by the Notch pathway, an important pathway in cell-cell communication and

cellular differentiation, which is affected when *Lkb1* is homozygously deleted in gastrointestinal epithelium (Shorning et al. 2009).

We also observed defects in the differentiation of secretory cells in intestinal polyps, suggesting the blockade of a common mechanism in the differentiation of these cell types. Such common denominators are the Notch signaling, which promotes proliferation and inhibits secretory cell differentiation both in the intestine and in the stomach (Kim and Shivdasani 2011; Kim and Shivdasani 2011), and the Notch-regulated transcription factor SAM pointed domain ETS factor (Spdef), which is driving the differentiation of secretory cells in both the stomach and the intestine (Gregorieff et al. 2009; Horst et al. 2010; Noah et al. 2010), and also preferentially driving the differentiation of goblet cells from goblet/Paneth cell precursors (Noah et al. 2010). Intestinal secretory cells also depend on the transcription factor Math1 (Yang et al. 2001), and Notch signaling also represses this transcription factor (Kim and Shivdasani 2011). However, Math1 is not expressed in the stomach (Yang et al. 2001); therefore, inhibition of Math1 is an unlikely cause for loss of secretory cell fate in this compartment.

We also explored the role in PJS polyposis of COX-2, an enzyme eminently attributed to control of the inflammatory reaction (Vane et al. 1994)(II, IV). Many aspects of the inflammatory reaction are beneficial to tumor cell growth, and cells associated with inflammation, like immune cells and myofibroblasts, as well as inflammation related signaling molecules, cytokines, are commonly present also in tumor stroma (Mantovani et al. 2008). Such an activated stroma has been shown to carry the potential to induce malignant transformation in epithelial cells (e.g. (Hayward et al. 2001)). Also, polypoid structures in the mucous membranes can be caused by inflammation, for instance during Helicobacter pylori infection as inflammation –associated hyperplastic polyps occur in the stomach and regress upon eradication of the bacteria (Ohkusa et al. 1998). Although these structures, *per se*, are unrelated to the genetically determined polyps of the Peutz-Jeghers syndrome, this highlights the potency of inflammatory signaling mediators in tumor and polyp formation.

In other gastrointestinal polyposes, and particularly in Familial Adenomatous Polyposis (FAP), the effects of COX-2 have been extensively studied, and COX-2 has been shown to correlate with increased adenoma size (Azumaya et al. 2002), higher proliferation (Sato et al. 2003) and a worse prognosis of colorectal adenomas and cancers (Han et al. 2010). COX-2 inhibition has also consistently been found to reduce the adenoma burden in both FAP patients(Steinbach et al. 2000), and in the $Apc^{Min/+}$ mouse model of the disease (Jacoby et al. 2000) through inhibition of angiogenesis and upregulation of apoptosis (Leahy et al. 2002).

In Peutz-Jeghers polyposis there is a dispute regarding where COX-2 is expressed, some results show epithelial expression (Rossi et al. 2002; De Leng et al. 2003), others

generalized stromal expression (Takeda et al. 2004). This could be due to a difference between patient (Rossi et al. 2002; De Leng et al. 2003) and mouse polyps (Takeda et al. 2004). Also a third, unexplored but potential source of COX-2 expression could be activated stromal immune cells, especially macrophages as shown in the $Apc^{Min/+}$ mice (Nakanishi et al. 2011). From the perspective of tumor progression, however, this ambiguity makes very little difference, as COX-2 could excert its effects on polyp progression as well from any of these locations, as demonstrated in Juvenile Polyposis (Brazowski et al. 2005).

Here, in studies II and IV, we did not dwell further on the localization of COX-2 expression, but rather studied the effects of ablation or inhibition of COX-2 on polyposis. We found COX-2 to promote the growth of polyps, rather than their initiation (II), which is consistent with observations from Familial Adenomatous Polyposis, where COX-2 is linked to later stages of polyp growth (Takeda et al. 2003). COX-2 inhibition by celecoxib did not increase the apoptosis frequency in the PJS polyp epithelium, and polyp numbers were not altered as much as the polyp burden in the $Lkb1^{+/-}$ mice, whereas in FAP, a reduction in polyp number was associated with an increase in the apoptotic ratio of adenomas in a subset of patients (Sinicrope et al. 2004). However, we found that COX-2 inhibition reduced the microvessel density, as a sign of reduced angiogenesis, which may limit the flow of nutrients to the polyp cells. Therefore, celecoxib treatment could potentially have caused the suppression of $Lkb1^{+/-}$ polyposis through decreased epithelial proliferation, as observed upon celecoxib treatment in FAP (Sinicrope et al. 2004). Such an effect could have been mediated both through the restriction of angiogenesis, as direct inhibition, or both. In study IV, in addition, COX-2 inhibition was not particularly effective in limiting the $Lkb1^{+/-}$ polyp growth, when initiation had been spurred by mutagenesis affecting also other genes than *Lkb1* (IV), suggesting this kind of growth increase was independent of COX-2 and dependent on some other factors.

It remains a subject for further study which genes can drive Peutz-Jeghers polyp initiation and progression in the *LKB1*-heterozygous mucosa, although focal accumulation of mutated p53 in a carcinogen treated $Lkb1^{+/-}$ polyp (IV) supported the notion that *p53* is one of them (Wei et al. 2005; Takeda et al. 2006). Interestingly, the additional mutations acquired by the $Lkb1^{+/-}$ epithelium in this study did not cause significant amounts of malignant transformation in the polyps, conforming to a previously rather debated finding, that *LKB1* loss drives benign growth but resistance to malignant transformation (Bardeesy et al. 2002).

This phenomenon of transformation resistance upon loss of *LKB1* has recently has been suggested to depend on impaired AMPK regulation of nicotinamide adenine dinucleotide phosphate (NADPH) homeostasis, disrupting the redox balance of cells growing under energy stress conditions typical for malignant growth (Jeon et al. 2012), so that toxic reactive oxygen species accumulate and eventually destroy the cell. Such a chain of

events has also been suggested to occur in hematopoietic stem cells upon *Lkb1* ablation (Gan et al. 2010; Gurumurthy et al. 2010; Nakada et al. 2010). In all other settings, however, where this intriguing effect has been observed, the cells have suffered a homozygous deletion of *LKB1*, whereas in the PJS polyps, both the epithelial and the stromal cells are heterozygous for *LKB1* loss. As it is not evident that a similar reactive oxygen species overload can arise also when some LKB1 function is retained, it remains unclear whether this mechanism protects against polyp transformation or not.

As a follow-up on the findings in studies III and IV and on other studies on the different roles of the stroma and epithelium in polyp formation in Peutz-Jeghers polyp formation (Katajisto et al. 2008; Shorning et al. 2009), further studies on the topic of epithelial-stromal interaction in the formation of polyps would be of future interest. Using mouse models, as in the studies presented here, such experimentation could be performed as sequential induction of *Lkb1* loss in the stroma and epithelium, by means of inducible *Cre* transgenes.

The *TagIn-Cre* mouse (Katajisto et al. 2008) is a good model for temporally controlled stromal *Lkb1* inactivation, but a similar model of gastric epithelial *Lkb1* knockout remains to be developed. The $Lgr5^{Cre\text{-}ERT2}$(Barker et al. 2007) could be used to study specifically the antral mucosa (Barker et al. 2010) or alternatively, intestinal PJS polyposis could be studied instead in this system or with the $villin^{Cre\text{-}ERT2}$ (el Marjou et al. 2004). The main reason for studying gastric polyps in the $Lkb1^{+/-}$ mouse model, has been the high frequency of polyps in the stomach (100% of mice) as compared to the intestine (5-10% of mice depending on background, our unpublished data). On the other hand, stydying intestinal polyposis would make sense from the point of view of Peutz-Jeghers patient symptoms, as intestinal polyps cause the bulk of polyp-related complications

The recent finding that stromal loss of *LKB1* can evoke endometrial carcinogenesis without manipulations of the epithelial compartment (Tanwar et al. 2012) also suggests that there is a need for the development of similar compartmentally regulated stromal vs. epithelial model systems for the study of other tissues subject to *LKB1*-regulated tumorigenesis (like the mammary gland (Shen et al. 2002) or lung (Sanchez-Cespedes et al. 2002)).

Considering the vast amount of LKB1 substrates and interactors that also could be explored in this manner, large-scale experimentation through some less time-consuming approach than the mouse models, perhaps systematic organoid culture combined with bioinformatical processing of data, could be applied as a starting point for clarifying the tissue and cell type specific functions of LKB1 in Peutz-Jeghers polyposis and beyond.

SUMMARY AND CONCLUSIONS

In conclusion, evaluating the impact of the findings of this thesis upon the research field, Study I has turned out highly relevant for the further studies of LKB1 signaling both *in vitro* and *in vivo*. This study, together with a previous report on AMPK activation by LKB1 (Hawley et al. 2003), and other research groups arriving at similar findings (Shaw et al. 2004) opened up whole new fields for research, as described above. Also the MEF cell lines engineered for study I have been used by other research groups to clarify specific cellular functions of AMPK and LKB1 (see e.g. (Sakamoto et al. 2004; Rattan et al. 2005; Memmott et al. 2008)).

In the case of study II, however, right after the publication of the study, concern was raised regarding the cardiovascular side-effects of COX-2 selective inhibitors, and closely related drug, rofecoxib, was withdrawn from the market, and thus no further clinical trials with celecoxib were pursued. The advancement of the findings from study II into any form of clinical practice would require more large scale clinical assessment of COX-2 inhibitor efficacy in the treatment of the disease before coming into question. As of yet, there has been no apparent interest in such studies from the side of the drug manufacturers, and currently mTOR inhibitors are the primary line of PJS treatment research being pursued in the clinics.

The cardiovascular side effects of coxibs have, however, since then proven to be of the same level as for most non-selective non-steroidal anti-inflammatory drugs (www.mhra.gov.uk/home/groups/plp/ documents/websiteresources/con068576.pdf), and the future of COX-2 inhibitor therapy in PJS is still not determined. Treatment with COX-2 inhibitors is accepted as standard protocol for patients suffering from Familial Adenomatous polyposis (Phillips et al. 2002). Also in other forms of cancer, the efficacy of COX-2 inhibitors, primarily celecoxib, have been widely addressed. There are, for example, phase II trials in head-and neck cancer (Mohammadianpanah et al. 2012), ovarian cancer (Reyners et al. 2012) and breast cancer (Young et al. 2012) that recently have been reported successful. Insomuch that at the time being, COX-2 inhibition is seen as a significant tool in fighting cancer in general (McCarty 2011). Thus, a new stance to COX-2 inhibitor treatment in PJS may well be taken in the future. The treatment could, potentially, be offered in combination with other medical and surgical treatments, rather than on its own.

The findings of study III, in turn, may increase our future understanding of glandular cancer development in Peutz-Jeghers disease and perhaps also beyond. Kato et. al (Kato et al. 2011) recently reported widespread pseudo-pyloric metaplasia in a PJS patient, calling for re-evaluation of previously studied polyp and tumor series from PJS patients

for this type of lesions, and suggesting similar LKB1-linked deregulation of glandular cell differentiation also outside the stomach.

In study IV, as we treated *Lkb1*$^{+/-}$ mice with the MNU carcinogen to achieve additional DNA damage, we did not observe any substantial neoplastic response in the *Lkb1*$^{+/-}$ polyps, supporting the notion that *LKB1* loss drives benign growth but resistance to malignant transformation (Bardeesy et al. 2002). On the other hand, the initiation of Peutz-Jeghers polyps was dramatically increased. Study IV thus suggests *LKB1* heterozygosity may cooperate with mutations in other tumor suppressors and oncogenes in the formation of Peutz-Jeghers polyps, rather than this process being purely *LKB1*-dependent.

In the future, characterization of the functions of each of the separate LKB1 substrates will be required to grasp the system of LKB1 regulated signaling circuits. Considering also the vast amount of other potential interacting partners in LKB1 signaling and the combinations of *LKB1* loss with mutations in other genes as well as the roles of these substrates and interactors in different tissues, the characterization of LKB1 signaling has only begun. This research field will continue to require the combined efforts of research groups, on a large scale, to reach understanding of the Peutz-Jeghers syndrome and of LKB1 function.

ACKNOWLEDGEMENTS

This work was carried out within the Molecular Cancer Biology and the Translational Genome-Scale Biology research programs, at the Department of Biochemistry and Developmental Biology of the Institute of Biomedicine, and at the Institute of Biotechnology of the University of Helsinki. I would wish to acknowledge Olli Jänne, Esa Korpi, Hannu Sariola and Tomi Mäkelä, who provided these excellent facilities to work in, as well as Lauri Aaltonen, Kari Alitalo and Jorma Keski-Oja for the collaborative and supportive atmosphere of the research programs.

The work was undertaken with support from the MD/PhD Program of the Faculty of Medicine at the University of Helsinki associated with the Helsinki Biomedical Graduate Program (HBGP). I would like to express my gratitude for the financial and educational opportunities both in Helsinki and abroad offered through this program, and thank all past and present HBGP staff for their assistance. Financial support for this work was also received from the Finnish cancer foundation, the Biomedicum Helsinki foundation, the Maud Kuistila foundation, the Finnish Medical foundation, the University of Helsinki foundation, the Finska Läkaresällkapet foundation, K. Albin Johansson foundation, WWPeltonen foundation of Åbo Akademi, the Stockmann foundation and the Liv och Hälsa foundation, for which I express my most sincere gratitude.

Foremost, I would wish to acknowledge my thesis supervisor professor Tomi Mäkelä, who accepted me into his research group, for providing research facilities, resources, and contacts, that have offered a next to unlimited basis for the studies, for the enthusiasm and spirit of his research group, and for his critical approach to literature, which has taught me a lot. I would also like to thank professor Mäkelä together with professors Ari Ristimäki and Matti Airaksinen for their support as members of my thesis follow-up committee. Thank you guys for not giving up on me!

Pre-examiners Pauli Puolakkainen and Tatiana Petrova are gratefully acknowledged for reviewing the thesis manuscript on a very tight schedule, for their gentle comments and suggestions to the manuscript, as well as for prepping me for the dissertation. Dear Emma Murros is thanked for her expert help with the English language.

I would further like to thank all my collaborators in the studies, Pekka Katajisto, Derrick Rossi, Anna Lepistö, Anna-Maria Lahesmaa, Marika Kyyrönen, Antti Ylikorkala, Heikki Järvinen, Ari Ristimäki, and Tomi Mäkelä in Helsinki and Jose Lizcano, Olga Göransson, Rachel Toth, Maria Deak, Nick Morrice, Jérôme Boudeau, and Simon Hawley, from the groups of Dario Alessi and Grahame Hardie in Dundee, without whom none of the work included in this thesis would have been possible.

I would also like to thank all other past and present colleagues in the Mäkelä group. A special thanks goes to Derrick, Birgitta, Marianne, Anou, Saara, Antti, Tea, Pekka, Thomas, Katja, Kari, Nina and Marie for all the important advice and critical comments to my research, and to Eeva, Ying, Katja, Kaisa, Tima, Ana-Maria, Jimmy, Jing, Bianca, and Emma for the fun times. Birgitta, Outi, Susanna, Kirsi and Saana are thanked both for the technical assistance they have contributed with during this thesis work, as well as for their kindness and contribution to the lab atmosphere. Also, I would wish to thank Satu Sankkila, Ritva Lautala, Aija Kaitera, Paula Turkkelin and Tapio Tainola for guiding my way through both bureaucracy and practical matters.

I also wish to thank Päivi Ojala, Juha Klefström and their labs for all the joint experiences both scientifically and otherwise, especially Johanna, Anni, Annika & Annika and Gregorz. And, of course, I also wish to express my thankfulness to all you other members of the Cancer Biology research program and Genome Scale Biology research program for the fruitful scientific discussions and all the fun times we have had together. Also, a big thank you to all of you HBGP student council members with whom we went through fire and storms arranging both great scientific meetings and great parties. I will miss you all – *"ingen nämnd och ingen glömd"*.

Also, I would like to thank all my past supervisors, who helped raise my interest in research and perspective on the scientific method: Carina Wallgren-Pettersson, Ralf Krahe, Leena Peltonen, Maija Wessman, Markku Heikinheimo and Raili Kauppinen. I wish to thank also my past teachers in mathematics, Roland Eklund, and chemistry, Stefan Röj, who did not accept my study choices for high-school and decided to convince me to scrap my artistic-linguistic plans and concentrate on the natural sciences.

Last but not least, I wish to thank my family, my father Bjarne, who got me there into that first research lab at age 17, and my mother, Ursula, who was there for my son when this thesis was being written. For all strength, hope, and dreams I want to thank my husband Simo and our son Walter, may you live your dreams too!

Helsinki 24.10.2012

Lina Udd

REFERENCES

*Alhopuro, P., P. Katajisto, R. Lehtonen, S. K. Ylisaukko-Oja, L. Naatsaari, A. Karhu, A. M. Westerman, J. H. Wilson, F. W. de Rooij, T. Vogel, G. Moeslein, I. P. Tomlinson, L. A. Aaltonen, T. P. Makela and V. Launonen (2005). "Mutation analysis of three genes encoding novel LKB1-interacting proteins, BRG1, STRADalpha, and MO25alpha, in Peutz-Jeghers syndrome." Br J Cancer **92**(6): 1126-9.*

*Alvarado-Kristensson, M., M. J. Rodriguez, V. Silio, J. M. Valpuesta and A. C. Carrera (2009). "SADB phosphorylation of gamma-tubulin regulates centrosome duplication." Nat Cell Biol **11**(9): 1081-92.*

*Andreelli, F., M. Foretz, C. Knauf, P. D. Cani, C. Perrin, M. A. Iglesias, B. Pillot, A. Bado, F. Tronche, G. Mithieux, S. Vaulont, R. Burcelin and B. Viollet (2006). "Liver adenosine monophosphate-activated kinase-alpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin." Endocrinology **147**(5): 2432-41.*

*Aretz, S., D. Stienen, S. Uhlhaas, S. Loff, W. Back, C. Pagenstecher, D. R. McLeod, G. E. Graham, E. Mangold, R. Santer, P. Propping and W. Friedl (2005). "High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome." Hum Mutat **26**(6): 513-9.*

*Asada, N. and K. Sanada (2010). "LKB1-mediated spatial control of GSK3beta and adenomatous polyposis coli contributes to centrosomal forward movement and neuronal migration in the developing neocortex." J Neurosci **30**(26): 8852-65.*

*Asada, N., K. Sanada and Y. Fukada (2007). "LKB1 regulates neuronal migration and neuronal differentiation in the developing neocortex through centrosomal positioning." J Neurosci **27**(43): 11769-75.*

*Avizienyte, E., A. Loukola, S. Roth, A. Hemminki, M. Tarkkanen, R. Salovaara, J. Arola, R. Butzow, K. Husgafvel-Pursiainen, A. Kokkola, H. Jarvinen and L. A. Aaltonen (1999). "LKB1 somatic mutations in sporadic tumors." Am J Pathol **154**(3): 677-81.*

*Avizienyte, E., S. Roth, A. Loukola, A. Hemminki, R. A. Lothe, A. E. Stenwig, S. D. Fossa, R. Salovaara and L. A. Aaltonen (1998). "Somatic mutations in LKB1 are rare in sporadic colorectal and testicular tumors." Cancer Res **58**(10): 2087-90.*

*Azumaya, M., M. Kobayashi, Y. Ajioka, T. Honma, Y. Suzuki, M. Takeuchi, R. Narisawa and H. Asakura (2002). "Size-dependent expression of cyclooxygenase-2 in sporadic colorectal adenomas relative to adenomas in patients with familial adenomatous polyposis." Pathol Int **52**(4): 272-6.*

*Baas, A. F., J. Boudeau, G. P. Sapkota, L. Smit, R. Medema, N. A. Morrice, D. R. Alessi and H. C. Clevers (2003). "Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD." EMBO J **22**(12): 3062-72.*

*Baas, A. F., J. Kuipers, N. N. van der Wel, E. Batlle, H. K. Koerten, P. J. Peters and H. C. Clevers (2004). "Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD." Cell **116**(3): 457-66.*

*Bardeesy, N., M. Sinha, A. F. Hezel, S. Signoretti, N. A. Hathaway, N. E. Sharpless, M. Loda, D. R. Carrasco and R. A. DePinho (2002). "Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation." Nature **419**(6903): 162-7.*

*Barker, N., M. Huch, P. Kujala, M. van de Wetering, H. J. Snippert, J. H. van Es, T. Sato, D. E. Stange, H. Begthel, M. van den Born, E. Danenberg, S. van den Brink, J. Korving, A. Abo, P. J. Peters, N. Wright, R. Poulsom and H. Clevers (2010). "Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro." Cell Stem Cell **6**(1): 25-36.*

*Barker, N., J. H. van Es, J. Kuipers, P. Kujala, M. van den Born, M. Cozijnsen, A. Haegebarth, J. Korving, H. Begthel, P. J. Peters and H. Clevers (2007). "Identification of stem cells in small intestine and colon by marker gene Lgr5." Nature **449**(7165): 1003-7.*

*Barnes, A. P., B. N. Lilley, Y. A. Pan, L. J. Plummer, A. W. Powell, A. N. Raines, J. R. Sanes and F. Polleux (2007). "LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons." Cell **129**(3): 549-63.*

*Beghini, A., I. Magnani, G. Roversi, T. Piepoli, S. Di Terlizzi, R. F. Moroni, B. Pollo, A. M. Fuhrman Conti, J. K. Cowell, G. Finocchiaro and L. Larizza (2003). "The neural progenitor-restricted isoform of the MARK4 gene in 19q13.2 is upregulated in human gliomas and overexpressed in a subset of glioblastoma cell lines." Oncogene **22**(17): 2581-91.*

*Bessone, S., F. Vidal, Y. Le Bouc, J. Epelbaum, M. T. Bluet-Pajot and M. Darmon (1999). "EMK protein kinase-null mice: dwarfism and hypofertility associated with alterations in the somatotrope and prolactin pathways." Dev Biol **214**(1): 87-101.*

*Bignell, G. R., R. Barfoot, S. Seal, N. Collins, W. Warren and M. R. Stratton (1998). "Low frequency of somatic mutations in the LKB1/Peutz-Jeghers syndrome gene in sporadic breast cancer." Cancer Res **58**(7): 1384-6.*

*Blache, P., M. van de Wetering, I. Duluc, C. Domon, P. Berta, J. N. Freund, H. Clevers and P. Jay (2004). "SOX9 is an intestine crypt transcription factor, is regulated by the Wnt pathway, and represses the CDX2 and MUC2 genes." J Cell Biol **166**(1): 37-47.*

*Boudeau, J., A. F. Baas, M. Deak, N. A. Morrice, A. Kieloch, M. Schutkowski, A. R. Prescott, H. C. Clevers and D. R. Alessi (2003b). "MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm." EMBO J **22**(19): 5102-14.*

*Boudeau, J., M. Deak, M. A. Lawlor, N. A. Morrice and D. R. Alessi (2003a). "Heat-shock protein 90 and Cdc37 interact with LKB1 and regulate its stability." Biochem J **370**(Pt 3): 849-57.*

*Boudeau, J., J. W. Scott, N. Resta, M. Deak, A. Kieloch, D. Komander, D. G. Hardie, A. R. Prescott, D. M. van Aalten and D. R. Alessi (2004). "Analysis of the LKB1-STRAD-MO25 complex." J Cell Sci **117**(Pt 26): 6365-75.*

*Brazowski, E., P. Rozen, F. Misonzhnick-Bedny and G. Gitstein (2005). "Characteristics of familial juvenile polyps expressing cyclooxygenase-2." Am J Gastroenterol **100**(1): 130-8.*

*Bright, N. J., C. Thornton and D. Carling (2009). "The regulation and function of mammalian AMPK-related kinases." Acta Physiol (Oxf) **196**(1): 15-26.*

*Brown, J. D., C. R. Hancock, A. D. Mongillo, J. Benjamin Barton, R. A. DiGiovanni, A. C. Parcell, W. W. Winder and D. M. Thomson (2011). "Effect of LKB1 deficiency on mitochondrial content, fibre type and muscle performance in the mouse diaphragm." Acta Physiol (Oxf) **201**(4): 457-66.*

*Brugarolas, J. and W. G. Kaelin, Jr. (2004). "Dysregulation of HIF and VEGF is a unifying feature of the familial hamartoma syndromes." Cancer Cell **6**(1): 7-10.*

*Buchet-Poyau, K., H. Mehenni, U. Radhakrishna and S. E. Antonarakis (2002). "Search for the second Peutz-Jeghers syndrome locus: exclusion of the STK13, PRKCG, KLK10, and PSCD2 genes on chromosome 19 and the STK11IP gene on chromosome 2." Cytogenet Genome Res **97**(3-4): 171-8.*

*Buler, M., S. M. Aatsinki, R. Skoumal, Z. Komka, M. Toth, R. Kerkela, A. Georgiadi, S. Kersten and J. Hakkola (2011). "Energy-sensing factors coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha) and AMP-activated protein kinase control expression of inflammatory mediators in liver: induction of interleukin 1 receptor antagonist." J Biol Chem **287**(3): 1847-60.*

*Cao, Y., H. Li, H. Liu, C. Zheng, H. Ji and X. Liu (2010). "The serine/threonine kinase LKB1 controls thymocyte survival through regulation of AMPK activation and Bcl-XL expression." Cell Res **20**(1): 99-108.*

*Carling, D., P. R. Clarke, V. A. Zammit and D. G. Hardie (1989). "Purification and characterization of the AMP-activated protein kinase. Copurification of acetyl-CoA carboxylase kinase and 3-hydroxy-3-methylglutaryl-CoA reductase kinase activities." Eur J Biochem **186**(1-2): 129-36.*

*Carretero, J., T. Shimamura, K. Rikova, A. L. Jackson, M. D. Wilkerson, C. L. Borgman, M. S. Buttarazzi, B. A. Sanofsky, K. L. McNamara, K. A. Brandstetter, Z. E. Walton, T. L. Gu, J. C. Silva, K. Crosby, G. I. Shapiro, S. M. Maira, H. Ji, D. H. Castrillon, C. F. Kim, C. Garcia-Echeverria, N. Bardeesy, N. E. Sharpless, N. D. Hayes, W. Y. Kim, J. A. Engelman and K. K. Wong (2010). "Integrative genomic and proteomic analyses identify targets for Lkb1-deficient metastatic lung tumors." Cancer Cell **17**(6): 547-59.*

*Chaiyapan, W., S. Sangkhathat, S. Kanngurn, M. Phukaoloun, P. Chiengkriwate and S. Patrapinyokul (2010). "Immunohistological evidence for Wnt-signaling activation in Peutz-Jeghers polyposis." Pediatr Surg Int **26**(2): 173-7.*

*Chen, Z., K. Cheng, Z. Walton, Y. Wang, H. Ebi, T. Shimamura, Y. Liu, T. Tupper, J. Ouyang, J. Li, P. Gao, M. S. Woo, C. Xu, M. Yanagita, A. Altabef, S. Wang, C. Lee, Y. Nakada, C. G. Pena, Y. Sun, Y. Franchetti, C. Yao, A. Saur, M. D. Cameron, M. Nishino, D. N. Hayes, M. D. Wilkerson, P. J. Roberts, C. B. Lee, N. Bardeesy, M. Butaney, L. R. Chirieac, D. B. Costa, D. Jackman, N. E. Sharpless, D. H. Castrillon, G. D. Demetri, P. A. Janne, P. P. Pandolfi, L. C. Cantley, A. L. Kung, J. A. Engelman and K. K. Wong (2010). "A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response." Nature **483**(7391): 613-7.*

*Cheng, H., P. Liu, Z. C. Wang, L. Zou, S. Santiago, V. Garbitt, O. V. Gjoerup, J. D. Iglehart, A. Miron, A. L. Richardson, W. C. Hahn and J. J. Zhao (2009). "SIK1 couples LKB1 to p53-dependent anoikis and suppresses metastasis." Sci Signal **2**(80): ra35.*

*Chun, C. Z., S. Kaur, G. V. Samant, L. Wang, K. Pramanik, M. K. Garnaas, K. Li, L. Field, D. Mukhopadhyay and R. Ramchandran (2009). "Snrk-1 is involved in multiple steps of angioblast development and acts via notch signaling pathway in artery-vein specification in vertebrates." Blood **113**(5): 1192-9.*

*Claret, M., M. A. Smith, C. Knauf, H. Al-Qassab, A. Woods, A. Heslegrave, K. Piipari, J. J. Emmanuel, A. Colom, P. Valet, P. D. Cani, G. Begum, A. White, P. Mucket, M. Peters, K. Mizuno, R. L. Batterham, K. P. Giese, A. Ashworth, R. Burcelin, M. L. Ashford, D. Carling and D. J. Withers (2011). "Deletion of Lkb1 in pro-opiomelanocortin neurons impairs peripheral glucose homeostasis in mice." Diabetes **60**(3): 735-45.*

*Collins, S. P., J. L. Reoma, D. M. Gamm and M. D. Uhler (2000). "LKB1, a novel serine/threonine protein kinase and potential tumour suppressor, is phosphorylated by cAMP-dependent protein kinase (PKA) and prenylated in vivo." Biochem J **345 Pt 3**: 673-80.*

*Contreras, C. M., E. A. Akbay, T. D. Gallardo, J. M. Haynie, S. Sharma, O. Tagao, N. Bardeesy, M. Takahashi, J. Settleman, K. K. Wong and D. H. Castrillon (2010). "Lkb1 inactivation is sufficient to drive endometrial cancers that are aggressive yet highly responsive to mTOR inhibitor monotherapy." Dis Model Mech **3**(3-4): 181-93.*

*Contreras, C. M., S. Gurumurthy, J. M. Haynie, L. J. Shirley, E. A. Akbay, S. N. Wingo, J. O. Schorge, R. R. Broaddus, K. K. Wong, N. Bardeesy and D. H. Castrillon (2008). "Loss of Lkb1 provokes highly invasive endometrial adenocarcinomas." Cancer Res **68**(3): 759-66.*

*Corradetti, M. N., K. Inoki, N. Bardeesy, R. A. DePinho and K. L. Guan (2004). "Regulation of the TSC pathway by LKB1: evidence of a molecular link between tuberous sclerosis complex and Peutz-Jeghers syndrome." Genes Dev **18**(13): 1533-8.*

*Dale, S., W. A. Wilson, A. M. Edelman and D. G. Hardie (1995). "Similar substrate recognition motifs for mammalian AMP-activated protein kinase, higher plant HMG-CoA reductase kinase-A, yeast SNF1, and mammalian calmodulin-dependent protein kinase I." FEBS Lett **361**(2-3): 191-5.*

*Daley, W. P., E. M. Gervais, S. W. Centanni, K. M. Gulfo, D. A. Nelson and M. Larsen (2012). "ROCK1-directed basement membrane positioning coordinates epithelial tissue polarity." Development **139**(2): 411-22.*

*Davies, S. P., N. R. Helps, P. T. Cohen and D. G. Hardie (1995). "5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC." FEBS Lett **377**(3): 421-5.*

*de Leng, W. W., M. Jansen, R. Carvalho, M. Polak, A. R. Musler, A. N. Milne, J. J. Keller, F. H. Menko, F. W. de Rooij, C. A. Iacobuzio-Donahue, F. M. Giardiello, M. A. Weterman and G. J. Offerhaus (2007b). "Genetic defects underlying Peutz-Jeghers syndrome (PJS) and exclusion of the polarity-associated MARK/Par1 gene family as potential PJS candidates." Clin Genet **72**(6): 568-73.*

*de Leng, W. W., M. Jansen, J. J. Keller, M. de Gijsel, A. N. Milne, F. H. Morsink, M. A. Weterman, C. A. Iacobuzio-Donahue, H. C. Clevers, F. M. Giardiello and G. J. Offerhaus (2007c). "Peutz-Jeghers syndrome polyps are polyclonal with expanded progenitor cell compartment." Gut **56**(10): 1475-6.*

*De Leng, W. W., A. M. Westerman, M. A. Weterman, F. W. De Rooij, H. Dekken Hv, A. F. De Goeij, S. B. Gruber, J. H. Wilson, G. J. Offerhaus, F. M. Giardiello and J. J. Keller (2003). "Cyclooxygenase 2 expression and molecular alterations in Peutz-Jeghers hamartomas and carcinomas." Clin Cancer Res **9**(8): 3065-72.*

*de Leng, W. W., A. M. Westerman, M. A. Weterman, M. Jansen, H. van Dekken, F. M. Giardiello, F. W. de Rooij, J. H. Paul Wilson, G. J. Offerhaus and J. J. Keller (2007a). "Nasal polyposis in Peutz-Jeghers syndrome: a distinct histopathological and molecular genetic entity." J Clin Pathol **60**(4): 392-6.*

*Denhardt, D. T., D. R. Edwards, M. McLeod, G. Norton, C. L. Parfett and M. Zimmer (1991). "Spontaneous immortalization of mouse embryo cells: strain differences and changes in gene expression with particular reference to retroviral gag-pol genes." Exp Cell Res **192**(1): 128-36.*

*Denison, F. C., N. J. Hiscock, D. Carling and A. Woods (2009). "Characterization of an alternative splice variant of LKB1." J Biol Chem **284**(1): 67-76.*

*Denison, F. C., L. B. Smith, P. J. Muckett, L. O'Hara, D. Carling and A. Woods (2010). "LKB1 is an essential regulator of spermatozoa release during spermiation in the mammalian testis." PLoS One **6**(12): e28306.*

*Dinchuk, J. E., B. D. Car, R. J. Focht, J. J. Johnston, B. D. Jaffee, M. B. Covington, N. R. Contel, V. M. Eng, R. J. Collins, P. M. Czerniak and et al. (1995). "Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II." Nature **378**(6555): 406-9.*

*Dorfman, J. and I. G. Macara (2008). "STRADalpha regulates LKB1 localization by blocking access to importin-alpha, and by association with Crm1 and exportin-7." Mol Biol Cell **19**(4): 1614-26.*

*Egan, D. F., D. B. Shackelford, M. M. Mihaylova, S. Gelino, R. A. Kohnz, W. Mair, D. S. Vasquez, A. Joshi, D. M. Gwinn, R. Taylor, J. M. Asara, J. Fitzpatrick, A. Dillin, B. Viollet, M. Kundu, M. Hansen and R. J. Shaw (2011). "Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy." Science **331**(6016): 456-61.*

*el Marjou, F., K. P. Janssen, B. H. Chang, M. Li, V. Hindie, L. Chan, D. Louvard, P. Chambon, D. Metzger and S. Robine (2004). "Tissue-specific and inducible Cre-mediated recombination in the gut epithelium." Genesis **39**(3): 186-93.*

*Elia, G., R. Chinery, A. M. Hanby, R. Poulsom and N. A. Wright (1994). "The production and characterization of a new monoclonal antibody to the trefoil peptide human spasmolytic polypeptide." Histochem J **26**(8): 644-7.*

*Ellisen, L. W., N. Carlesso, T. Cheng, D. T. Scadden and D. A. Haber (2001). "The Wilms tumor suppressor WT1 directs stage-specific quiescence and differentiation of human hematopoietic progenitor cells." EMBO J **20**(8): 1897-909.*

Eneling, K., L. Brion, V. Pinto, M. J. Pinho, J. I. Sznajder, N. Mochizuki, K. Emoto, P. Soares-da-Silva and A. M. Bertorello (2012a). "Salt-inducible kinase 1 regulates E-cadherin expression and intercellular junction stability." FASEB J.

*Eneling, K., J. Chen, L. C. Welch, H. Takemori, J. I. Sznajder and A. M. Bertorello (2012b). "Salt-inducible kinase 1 is present in lung alveolar epithelial cells and regulates active sodium transport." Biochem Biophys Res Commun **409**(1): 28-33.*

*Eng, C., H. Hampel and A. de la Chapelle (2001). "Genetic testing for cancer predisposition." Annu Rev Med **52**: 371-400.*

*Entius, M. M., J. J. Keller, A. M. Westerman, B. P. van Rees, M. L. van Velthuysen, A. F. de Goeij, J. H. Wilson, F. M. Giardiello and G. J. Offerhaus (2001). "Molecular genetic alterations in hamartomatous polyps and carcinomas of patients with Peutz-Jeghers syndrome." J Clin Pathol **54**(2): 126-31.*

*Esteller, M., M. F. Fraga, M. Guo, J. Garcia-Foncillas, I. Hedenfalk, A. K. Godwin, J. Trojan, C. Vaurs-Barriere, Y. J. Bignon, S. Ramus, J. Benitez, T. Caldes, Y. Akiyama, Y. Yuasa, V. Launonen, M. J. Canal, R. Rodriguez, G. Capella, M. A. Peinado, A. Borg, L. A. Aaltonen, B. A. Ponder, S. B. Baylin and J. G. Herman (2001). "DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis." Hum Mol Genet **10**(26): 3001-7.*

*Estrada, R. and H. J. Spjut (1983). "Hamartomatous polyps in Peutz-Jeghers syndrome. A light-, histochemical, and electron-microscopic study." Am J Surg Pathol **7**(8): 747-54.*

*Fearon, E. R. and B. Vogelstein (1990). "A genetic model for colorectal tumorigenesis." Cell **61**(5): 759-67.*

*Fenton, H., B. Carlile, E. A. Montgomery, H. Carraway, J. Herman, F. Sahin, G. H. Su and P. Argani (2006). "LKB1 protein expression in human breast cancer." Appl Immunohistochem Mol Morphol **14**(2): 146-53.*

*Filippi, B. M., P. de los Heros, Y. Mehellou, I. Navratilova, R. Gourlay, M. Deak, L. Plater, R. Toth, E. Zeqiraj and D. R. Alessi (2011). "MO25 is a master regulator of SPAK/OSR1 and MST3/MST4/YSK1 protein kinases." EMBO J **30**(9): 1730-41.*

*Foretz, M., N. Ancellin, F. Andreelli, Y. Saintillan, P. Grondin, A. Kahn, B. Thorens, S. Vaulont and B. Viollet (2005). "Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver." Diabetes **54**(5): 1331-9.*

*Fu, A., A. C. Ng, C. Depatie, N. Wijesekara, Y. He, G. S. Wang, N. Bardeesy, F. W. Scott, R. M. Touyz, M. B. Wheeler and R. A. Screaton (2009). "Loss of Lkb1 in adult beta cells increases beta cell mass and enhances glucose tolerance in mice." Cell Metab **10**(4): 285-95.*

*Furihata, C., Y. Iwasaki, T. Sugimura, M. Tatematsu and M. Takahashi (1973). "Differentiation of pepsinogen-producing cells in the fundic and pyloric mucosa of developing rats." Cell Differ **2**(3): 179-89.*

*Gan, B., J. Hu, S. Jiang, Y. Liu, E. Sahin, L. Zhuang, E. Fletcher-Sananikone, S. Colla, Y. A. Wang, L. Chin and R. A. Depinho (2010). "Lkb1 regulates quiescence and metabolic homeostasis of haematopoietic stem cells." Nature **468**(7324): 701-4.*

*Gandhi, L., K. L. McNamara, D. Li, C. L. Borgman, U. McDermott, K. A. Brandstetter, R. F. Padera, L. R. Chirieac, J. E. Settleman and K. K. Wong (2009). "Sunitinib prolongs survival in genetically engineered mouse models of multistep lung carcinogenesis." Cancer Prev Res (Phila) **2**(4): 330-7.*

*Gao, Y., Q. Xiao, H. Ma, L. Li, J. Liu, Y. Feng, Z. Fang, J. Wu, X. Han, J. Zhang, Y. Sun, G. Wu, R. Padera, H. Chen, K. K. Wong, G. Ge and H. Ji (2010). "LKB1 inhibits lung cancer progression through lysyl oxidase and extracellular matrix remodeling." Proc Natl Acad Sci U S A **107**(44): 18892-7.*

*Garcia-Martinez, J. M., S. Wullschleger, G. Preston, S. Guichard, S. Fleming, D. R. Alessi and S. L. Duce (2011). "Effect of PI3K- and mTOR-specific inhibitors on spontaneous B-cell follicular lymphomas in PTEN/LKB1-deficient mice." Br J Cancer **104**(7): 1116-25.*

*Gaude, H., N. Aznar, A. Delay, A. Bres, K. Buchet-Poyau, C. Caillat, A. Vigouroux, C. Rogon, A. Woods, J. M. Vanacker, J. Hohfeld, C. Perret, P. Meyer, M. Billaud and C. Forcet (2012). "Molecular chaperone complexes with antagonizing activities regulate stability and activity of the tumor suppressor LKB1." Oncogene **31**(12): 1582-91.*

*Giardiello, F. M., J. D. Brensinger, A. C. Tersmette, S. N. Goodman, G. M. Petersen, S. V. Booker, M. Cruz-Correa and J. A. Offerhaus (2000). "Very high risk of cancer in familial Peutz-Jeghers syndrome." Gastroenterology **119**(6): 1447-53.*

*Goldenring, J. R. and S. Nomura (2006). "Differentiation of the gastric mucosa III. Animal models of oxyntic atrophy and metaplasia." Am J Physiol Gastrointest Liver Physiol **291**(6): G999-1004.*

*Gormand, A., E. Henriksson, K. Strom, T. E. Jensen, K. Sakamoto and O. Goransson (2011). "Regulation of AMP-activated protein kinase by LKB1 and CaMKK in adipocytes." J Cell Biochem **112**(5): 1364-75.*

*Granot, Z., A. Swisa, J. Magenheim, M. Stolovich-Rain, W. Fujimoto, E. Manduchi, T. Miki, J. K. Lennerz, C. J. Stoeckert, Jr., O. Meyuhas, S. Seino, M. A. Permutt, H. Piwnica-Worms, N. Bardeesy and Y. Dor (2009). "LKB1 regulates pancreatic beta cell size, polarity, and function." Cell Metab **10**(4): 296-308.*

*Gregorieff, A., D. E. Stange, P. Kujala, H. Begthel, M. van den Born, J. Korving, P. J. Peters and H. Clevers (2009). "The ets-domain transcription factor Spdef promotes maturation of goblet and paneth cells in the intestinal epithelium." Gastroenterology **137**(4): 1333-45 e1-3.*

*Gruber, S. B., M. M. Entius, G. M. Petersen, S. J. Laken, P. A. Longo, R. Boyer, A. M. Levin, U. J. Mujumdar, J. M. Trent, K. W. Kinzler, B. Vogelstein, S. R. Hamilton, M. H. Polymeropoulos, G. J. Offerhaus and F. M. Giardiello (1998). "Pathogenesis of adenocarcinoma in Peutz-Jeghers syndrome." Cancer Res **58**(23): 5267-70.*

*Guigas, B., N. Taleux, M. Foretz, D. Detaille, F. Andreelli, B. Viollet and L. Hue (2007). "AMP-activated protein kinase-independent inhibition of hepatic mitochondrial oxidative phosphorylation by AICA riboside." Biochem J **404**(3): 499-507.*

*Gurumurthy, S., A. F. Hezel, E. Sahin, J. H. Berger, M. W. Bosenberg and N. Bardeesy (2008). "LKB1 deficiency sensitizes mice to carcinogen-induced tumorigenesis." Cancer Res **68**(1): 55-63.*

*Gurumurthy, S., S. Z. Xie, B. Alagesan, J. Kim, R. Z. Yusuf, B. Saez, A. Tzatsos, F. Ozsolak, P. Milos, F. Ferrari, P. J. Park, O. S. Shirihai, D. T. Scadden and N. Bardeesy (2010). "The Lkb1 metabolic sensor maintains haematopoietic stem cell survival." Nature **468**(7324): 659-63.*

*Habets, D. D., W. A. Coumans, M. El Hasnaoui, E. Zarrinpashneh, L. Bertrand, B. Viollet, B. Kiens, T. E. Jensen, E. A. Richter, A. Bonen, J. F. Glatz and J. J. Luiken (2009). "Crucial role for LKB1 to AMPKalpha2 axis in the regulation of CD36-mediated long-chain fatty acid uptake into cardiomyocytes." Biochim Biophys Acta **1791**(3): 212-9.*

*Han, Y. D., Y. K. Hong, J. G. Kang, Y. J. Choi and C. H. Park (2010). "Relation of the expression of cyclooxygenase-2 in colorectal adenomas and adenocarcinomas to angiogenesis and prognosis." J Korean Soc Coloproctol **26**(5): 339-46.*

*Hardie, D. G. and D. Carling (1997). "The AMP-activated protein kinase--fuel gauge of the mammalian cell?" Eur J Biochem **246**(2): 259-73.*

*Hawley, S. A., J. Boudeau, J. L. Reid, K. J. Mustard, L. Udd, T. P. Makela, D. R. Alessi and D. G. Hardie (2003). "Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade." J Biol **2**(4): 28.*

*Hayward, S. W., Y. Wang, M. Cao, Y. K. Hom, B. Zhang, G. D. Grossfeld, D. Sudilovsky and G. R. Cunha (2001). "Malignant transformation in a nontumorigenic human prostatic epithelial cell line." Cancer Res **61**(22): 8135-42.*

*Hearle, N., V. Schumacher, F. H. Menko, S. Olschwang, L. A. Boardman, J. J. Gille, J. J. Keller, A. M. Westerman, R. J. Scott, W. Lim, J. D. Trimbath, F. M. Giardiello, S. B. Gruber, G. J. Offerhaus, F. W. de Rooij, J. H. Wilson, A. Hansmann, G. Moslein, B. Royer-Pokora, T. Vogel, R. K. Phillips, A. D. Spigelman and R. S. Houlston (2006a). "Frequency and spectrum of cancers in the Peutz-Jeghers syndrome." Clin Cancer Res **12**(10): 3209-15.*

*Hearle, N. C., M. F. Rudd, W. Lim, V. Murday, A. G. Lim, R. K. Phillips, P. W. Lee, J. O'Donohue, P. J. Morrison, A. Norman, S. V. Hodgson, A. Lucassen and R. S. Houlston (2006b). "Exonic STK11 deletions are not a rare cause of Peutz-Jeghers syndrome." J Med Genet **43**(4): e15.*

*Hegedus, B., B. Dasgupta, J. E. Shin, R. J. Emnett, E. K. Hart-Mahon, L. Elghazi, E. Bernal-Mizrachi and D. H. Gutmann (2007). "Neurofibromatosis-1 regulates neuronal and glial cell differentiation from neuroglial progenitors in vivo by both cAMP- and Ras-dependent mechanisms." Cell Stem Cell **1**(4): 443-57.*

*Hezel, A. F., S. Gurumurthy, Z. Granot, A. Swisa, G. C. Chu, G. Bailey, Y. Dor, N. Bardeesy and R. A. Depinho (2008). "Pancreatic LKB1 deletion leads to acinar polarity defects and cystic neoplasms." Mol Cell Biol **28**(7): 2414-25.*

*Horike, N., A. Kumagai, Y. Shimono, T. Onishi, Y. Itoh, T. Sasaki, K. Kitagawa, O. Hatano, H. Takagi, T. Susumu, H. Teraoka, K. Kusano, Y. Nagaoka, H. Kawahara and H. Takemori (2010). "Downregulation of SIK2 expression promotes the melanogenic program in mice." Pigment Cell Melanoma Res **23**(6): 809-19.*

*Horst, D., X. Gu, M. Bhasin, Q. Yang, M. Verzi, D. Lin, M. Joseph, X. Zhang, W. Chen, Y. P. Li, R. A. Shivdasani and T. A. Libermann (2010). "Requirement of the epithelium-specific Ets transcription factor Spdef for mucous gland cell function in the gastric antrum." J Biol Chem **285**(45): 35047-55.*

*Hou, X., J. E. Liu, W. Liu, C. Y. Liu, Z. Y. Liu and Z. Y. Sun (2011). "A new role of NUAK1: directly phosphorylating p53 and regulating cell proliferation." Oncogene **30**(26): 2933-42.*

*Howard, T. A., D. N. Misra, M. Grove, M. J. Becich, J. S. Shao, M. Gordon and D. H. Alpers (1996). "Human gastric intrinsic factor expression is not restricted to parietal cells." J Anat **189 (Pt 2)**: 303-13.*

*Hu, P. J., J. Yu, Z. R. Zeng, W. K. Leung, H. L. Lin, B. D. Tang, A. H. Bai and J. J. Sung (2004). "Chemoprevention of gastric cancer by celecoxib in rats." Gut **53**(2): 195-200.*

*Huang, X., S. Wullschleger, N. Shpiro, V. A. McGuire, K. Sakamoto, Y. L. Woods, W. McBurnie, S. Fleming and D. R. Alessi (2008). "Important role of the LKB1-AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice." Biochem J **412**(2): 211-21.*

Hurov, J. B., M. Huang, L. S. White, J. Lennerz, C. S. Choi, Y. R. Cho, H. J. Kim, J. L. Prior, D. Piwnica-Worms, L. C. Cantley, J. K. Kim, G. I. Shulman and H. Piwnica-Worms (2007). "Loss of the Par-

*1b/MARK2 polarity kinase leads to increased metabolic rate, decreased adiposity, and insulin hypersensitivity in vivo." Proc Natl Acad Sci U S A **104**(13): 5680-5.*

*Ichinoseki-Sekine, N., H. Naito, K. Tsuchihara, I. Kobayashi, Y. Ogura, R. Kakigi, M. Kurosaka, R. Fujioka and H. Esumi (2009). "Provision of a voluntary exercise environment enhances running activity and prevents obesity in Snark-deficient mice." Am J Physiol Endocrinol Metab **296**(5): E1013-21.*

*Ikeda, Y., K. Sato, D. R. Pimentel, F. Sam, R. J. Shaw, J. R. Dyck and K. Walsh (2009). "Cardiac-specific deletion of LKB1 leads to hypertrophy and dysfunction." J Biol Chem **284**(51): 35839-49.*

*Jacoby, R. F., K. Seibert, C. E. Cole, G. Kelloff and R. A. Lubet (2000). "The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis." Cancer Res **60**(18): 5040-4.*

*Jaleel, M., A. McBride, J. M. Lizcano, M. Deak, R. Toth, N. A. Morrice and D. R. Alessi (2005). "Identification of the sucrose non-fermenting related kinase SNRK, as a novel LKB1 substrate." FEBS Lett **579**(6): 1417-23.*

*Jansen, M., W. W. de Leng, A. F. Baas, H. Myoshi, L. Mathus-Vliegen, M. M. Taketo, H. Clevers, F. M. Giardiello and G. J. Offerhaus (2006). "Mucosal prolapse in the pathogenesis of Peutz-Jeghers polyposis." Gut **55**(1): 1-5.*

*Jansen, M., J. P. Ten Klooster, G. J. Offerhaus and H. Clevers (2009). "LKB1 and AMPK family signaling: the intimate link between cell polarity and energy metabolism." Physiol Rev **89**(3): 777-98.*

*Jeghers, H., K. V. Mc and K. H. Katz (1949). "Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits; a syndrome of diagnostic significance." N Engl J Med **241**(26): 1031-6.*

*Jeon, S. M., N. S. Chandel and N. Hay (2012). "AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress." Nature **485**(7400): 661-5.*

*Jessen, N., H. J. Koh, C. D. Folmes, C. Wagg, N. Fujii, B. Lofgren, C. M. Wolf, C. I. Berul, M. F. Hirshman, G. D. Lopaschuk and L. J. Goodyear (2010). "Ablation of LKB1 in the heart leads to energy deprivation and impaired cardiac function." Biochim Biophys Acta **1802**(7-8): 593-600.*

*Ji, H., M. R. Ramsey, D. N. Hayes, C. Fan, K. McNamara, P. Kozlowski, C. Torrice, M. C. Wu, T. Shimamura, S. A. Perera, M. C. Liang, D. Cai, G. N. Naumov, L. Bao, C. M. Contreras, D. Li, L. Chen, J. Krishnamurthy, J. Koivunen, L. R. Chirieac, R. F. Padera, R. T. Bronson, N. I. Lindeman, D. C. Christiani, X. Lin, G. I. Shapiro, P. A. Janne, B. E. Johnson, M. Meyerson, D. J. Kwiatkowski, D. H. Castrillon, N. Bardeesy, N. E. Sharpless and K. K. Wong (2007). "LKB1 modulates lung cancer differentiation and metastasis." Nature **448**(7155): 807-10.*

*Jishage, K., J. Nezu, Y. Kawase, T. Iwata, M. Watanabe, A. Miyoshi, A. Ose, K. Habu, T. Kake, N. Kamada, O. Ueda, M. Kinoshita, D. E. Jenne, M. Shimane and H. Suzuki (2002). "Role of Lkb1, the causative gene of Peutz-Jegher's syndrome, in embryogenesis and polyposis." Proc Natl Acad Sci U S A **99**(13): 8903-8.*

Karuman, P., O. Gozani, R. D. Odze, X. C. Zhou, H. Zhu, R. Shaw, T. P. Brien, C. D. Bozzuto, D. Ooi, L. C. Cantley and J. Yuan (2001). "The Peutz-Jegher gene product LKB1 is a mediator of p53-dependent cell death." Mol Cell 7(6): 1307-19.

*Katajisto, P., K. Vaahtomeri, N. Ekman, E. Ventela, A. Ristimaki, N. Bardeesy, R. Feil, R. A. DePinho and T. P. Makela (2008). "LKB1 signaling in mesenchymal cells required for suppression of gastrointestinal polyposis." Nat Genet **40**(4): 455-9.*

*Kato, N., M. Sugawara, K. Maeda, N. Hosoya and T. Motoyama (2011). "Pyloric gland metaplasia/differentiation in multiple organ systems in a patient with Peutz-Jegher's syndrome." Pathol Int **61**(6): 369-72.*

*Katoh, Y., H. Takemori, X. Z. Lin, M. Tamura, M. Muraoka, T. Satoh, Y. Tsuchiya, L. Min, J. Doi, A. Miyauchi, L. A. Witters, H. Nakamura and M. Okamoto (2006). "Silencing the constitutive active transcription factor CREB by the LKB1-SIK signaling cascade." FEBS J **273**(12): 2730-48.*

*Kim, C. J., Y. G. Cho, J. Y. Park, T. Y. Kim, J. H. Lee, H. S. Kim, J. W. Lee, Y. H. Song, S. W. Nam, S. H. Lee, N. J. Yoo, J. Y. Lee and W. S. Park (2004). "Genetic analysis of the LKB1/STK11 gene in hepatocellular carcinomas." Eur J Cancer **40**(1): 136-41.*

*Kim, T. H. and R. A. Shivdasani (2011). "Genetic evidence that intestinal Notch functions vary regionally and operate through a common mechanism of Math1 repression." J Biol Chem **286**(13): 11427-33.*

*Kim, T. H. and R. A. Shivdasani (2011). "Notch signaling in stomach epithelial stem cell homeostasis." J Exp Med **208**(4): 677-88.*

*Kinzler, K. W. and B. Vogelstein (1998). "Landscaping the cancer terrain." Science **280**(5366): 1036-7.*

*Klumpen, H. J., K. C. Queiroz, C. A. Spek, C. J. van Noesel, H. C. Brink, W. W. de Leng, R. F. de Wilde, E. M. Mathus-Vliegen, G. J. Offerhaus, M. A. Alleman, A. M. Westermann and D. J. Richel (2011). "mTOR inhibitor treatment of pancreatic cancer in a patient With Peutz-Jeghers syndrome." J Clin Oncol **29**(6): e150-3.*

*Klutho, P. J., D. L. Costanzo-Garvey and R. E. Lewis (2011). "Regulation of glucose homeostasis by KSR1 and MARK2." PLoS One **6**(12): e29304.*

*Knudson, A. G., Jr. (1971). "Mutation and cancer: statistical study of retinoblastoma." Proc Natl Acad Sci U S A **68**(4): 820-3.*

*Koh, H. J., D. E. Arnolds, N. Fujii, T. T. Tran, M. J. Rogers, N. Jessen, Y. Li, C. W. Liew, R. C. Ho, M. F. Hirshman, R. N. Kulkarni, C. R. Kahn and L. J. Goodyear (2006). "Skeletal muscle-selective knockout of LKB1 increases insulin sensitivity, improves glucose homeostasis, and decreases TRB3." Mol Cell Biol **26**(22): 8217-27.*

*Koh, H. J., T. Toyoda, N. Fujii, M. M. Jung, A. Rathod, R. J. Middelbeek, S. J. Lessard, J. T. Treebak, K. Tsuchihara, H. Esumi, E. A. Richter, J. F. Wojtaszewski, M. F. Hirshman and L. J. Goodyear (2010). "Sucrose nonfermenting AMPK-related kinase (SNARK) mediates contraction-stimulated glucose transport in mouse skeletal muscle." Proc Natl Acad Sci U S A **107**(35): 15541-6.*

*Koivunen, P., M. Hirsila, A. M. Remes, I. E. Hassinen, K. I. Kivirikko and J. Myllyharju (2007). "Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF." J Biol Chem **282**(7): 4524-32.*

*Kowanetz, M., P. Lonn, M. Vanlandewijck, K. Kowanetz, C. H. Heldin and A. Moustakas (2008). "TGFbeta induces SIK to negatively regulate type I receptor kinase signaling." J Cell Biol **182**(4): 655-62.*

*Lai, C., J. Robinson, S. Clark, G. Stamp, R. Poulsom and A. Silver (2011). "Elevation of WNT5A expression in polyp formation in Lkb1+/- mice and Peutz-Jeghers syndrome." J Pathol **223**(5): 584-92.*

Langeveld, D., M. Jansen, D. V. de Boer, M. van Sprundel, L. A. Brosens, F. H. Morsink, F. M. Giardiello, G. J. Offerhaus and W. W. de Leng (2011). "Aberrant intestinal stem cell lineage dynamics in Peutz-Jeghers syndrome and familial adenomatous polyposis consistent with protracted clonal evolution in the crypt." Gut 61(6): 839-46.

*Lee, J. H., H. Koh, M. Kim, Y. Kim, S. Y. Lee, R. E. Karess, S. H. Lee, M. Shong, J. M. Kim, J. Kim and J. Chung (2007). "Energy-dependent regulation of cell structure by AMP-activated protein kinase." Nature **447**(7147): 1017-20.*

*Lee, J. H., H. Koh, M. Kim, J. Park, S. Y. Lee, S. Lee and J. Chung (2006). "JNK pathway mediates apoptotic cell death induced by tumor suppressor LKB1 in Drosophila." Cell Death Differ **13**(7): 1110-22.*

*Lennerz, J. K., J. B. Hurov, L. S. White, K. T. Lewandowski, J. L. Prior, G. J. Planer, R. W. t. Gereau, D. Piwnica-Worms, R. E. Schmidt and H. Piwnica-Worms (2010). "Loss of Par-1a/MARK3/C-TAK1 kinase leads to reduced adiposity, resistance to hepatic steatosis, and defective gluconeogenesis." Mol Cell Biol **30**(21): 5043-56.*

*Lin, T., C. Chao, S. Saito, S. J. Mazur, M. E. Murphy, E. Appella and Y. Xu (2005). "p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression." Nat Cell Biol **7**(2): 165-71.*

*Liu, Y., V. Poon, G. Sanchez-Watts, A. G. Watts, H. Takemori and G. Aguilera (2012). "Salt-inducible kinase is involved in the regulation of corticotropin-releasing hormone transcription in hypothalamic neurons in rats." Endocrinology **153**(1): 223-33.*

*Lizcano, J. M., O. Goransson, R. Toth, M. Deak, N. A. Morrice, J. Boudeau, S. A. Hawley, L. Udd, T. P. Makela, D. G. Hardie and D. R. Alessi (2004). "LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1." EMBO J **23**(4): 833-43.*

*Londesborough, A., K. Vaahtomeri, M. Tiainen, P. Katajisto, N. Ekman, T. Vallenius and T. P. Makela (2008). "LKB1 in endothelial cells is required for angiogenesis and TGFbeta-mediated vascular smooth muscle cell recruitment." Development **135**(13): 2331-8.*

*Ma, Y., G. Zhang, X. Fu, O. Xia, C. Zhan, L. Li, Z. Wang and B. Wu (2010). "Wnt signaling may be activated in a subset of Peutz-Jeghers syndrome polyps closely correlating to LKB1 expression." Oncol Rep **23**(6): 1569-76.*

*Mallory, S. B. and D. B. t. Stough (1987). "Genodermatoses with malignant potential." Dermatol Clin **5**(1): 221-30.*

*Manning, G., D. B. Whyte, R. Martinez, T. Hunter and S. Sudarsanam (2002). "The protein kinase complement of the human genome." Science **298**(5600): 1912-34.*

*Mantovani, A., P. Allavena, A. Sica and F. Balkwill (2008). "Cancer-related inflammation." Nature **454**(7203): 436-44.*

*Marignani, P. A., F. Kanai and C. L. Carpenter (2001). "LKB1 associates with Brg1 and is necessary for Brg1-induced growth arrest." J Biol Chem **276**(35): 32415-8.*

*Marignani, P. A., K. D. Scott, R. Bagnulo, D. Cannone, E. Ferrari, A. Stella, G. Guanti, C. Simone and N. Resta (2007). "Novel splice isoforms of STRADalpha differentially affect LKB1 activity, complex assembly and subcellular localization." Cancer Biol Ther **6**(10): 1627-31.*

*Martin, S. G. and D. St Johnston (2003). "A role for Drosophila LKB1 in anterior-posterior axis formation and epithelial polarity." Nature **421**(6921): 379-84.*

*Matenia, D., C. Hempp, T. Timm, A. Eikhof and E. M. Mandelkow (2012). "Microtubule affinity-regulating kinase 2 (MARK2) turns on phosphatase and tensin homolog (PTEN)-induced kinase 1 (PINK1) at Thr-313, a mutation site in Parkinson disease: effects on mitochondrial transport." J Biol Chem **287**(11): 8174-86.*

*McCarthy, A., C. J. Lord, K. Savage, A. Grigoriadis, D. P. Smith, B. Weigelt, J. S. Reis-Filho and A. Ashworth (2009). "Conditional deletion of the Lkb1 gene in the mouse mammary gland induces tumour formation." J Pathol **219**(3): 306-16.*

*McCarty, M. F. (2011). "Minimizing the cancer-promotional activity of cox-2 as a central strategy in cancer prevention." Med Hypotheses **78**(1): 45-57.*

*McGarrity, T. J., L. P. Peiffer, C. I. Amos, M. L. Frazier, M. G. Ward and M. K. Howett (2003). "Overexpression of cyclooxygenase 2 in hamartomatous polyps of Peutz-Jeghers syndrome." Am J Gastroenterol **98**(3): 671-8.*

*McGee, S. L., K. J. Mustard, D. G. Hardie and K. Baar (2008). "Normal hypertrophy accompanied by phosphoryation and activation of AMP-activated protein kinase alpha1 following overload in LKB1 knockout mice." J Physiol **586**(6): 1731-41.*

*Memmott, R. M., J. J. Gills, M. Hollingshead, M. C. Powers, Z. Chen, B. Kemp, A. Kozikowski and P. A. Dennis (2008). "Phosphatidylinositol ether lipid analogues induce AMP-activated protein kinase-dependent death in LKB1-mutant non small cell lung cancer cells." Cancer Res **68**(2): 580-8.*

*Miller, R. A., Q. Chu, J. Le Lay, P. E. Scherer, R. S. Ahima, K. H. Kaestner, M. Foretz, B. Viollet and M. J. Birnbaum (2011). "Adiponectin suppresses gluconeogenic gene expression in mouse hepatocytes independent of LKB1-AMPK signaling." J Clin Invest **121**(6): 2518-28.*

*Mills, J. C., N. Andersson, T. S. Stappenbeck, C. C. Chen and J. I. Gordon (2003). "Molecular characterization of mouse gastric zymogenic cells." J Biol Chem **278**(46): 46138-45.*

*Mills, J. C., A. J. Syder, C. V. Hong, J. L. Guruge, F. Raaii and J. I. Gordon (2001). "A molecular profile of the mouse gastric parietal cell with and without exposure to Helicobacter pylori." Proc Natl Acad Sci U S A **98**(24): 13687-92.*

*Miyaki, M., T. Iijima, K. Hosono, R. Ishii, M. Yasuno, T. Mori, M. Toi, T. Hishima, N. Shitara, K. Tamura, J. Utsunomiya, N. Kobayashi, T. Kuroki and T. Iwama (2000). "Somatic mutations of LKB1 and beta-catenin genes in gastrointestinal polyps from patients with Peutz-Jeghers syndrome." Cancer Res **60**(22): 6311-3.*

*Miyoshi, H., A. Deguchi, M. Nakau, Y. Kojima, A. Mori, M. Oshima, M. Aoki and M. M. Taketo (2009). "Hepatocellular carcinoma development induced by conditional beta-catenin activation in Lkb1+/- mice." Cancer Sci **100**(11): 2046-53.*

*Miyoshi, H., M. Nakau, T. O. Ishikawa, M. F. Seldin, M. Oshima and M. M. Taketo (2002). "Gastrointestinal hamartomatous polyposis in Lkb1 heterozygous knockout mice." Cancer Res **62**(8): 2261-6.*

*Mohammadianpanah, M., S. Razmjou-Ghalaei, A. Shafizad, Y. Ashouri-Taziani, B. Khademi, N. Ahmadloo, M. Ansari, S. Omidvari, A. Mosalaei and M. A. Mosleh-Shirazi (2012). "Efficacy and safety of concurrent chemoradiation with weekly cisplatin +/- low-dose celecoxib in locally advanced undifferentiated nasopharyngeal carcinoma: a phase II-III clinical trial." J Cancer Res Ther **7**(4): 442-7.*

*Morton, J. P., N. B. Jamieson, S. A. Karim, D. Athineos, R. A. Ridgway, C. Nixon, C. J. McKay, R. Carter, V. G. Brunton, M. C. Frame, A. Ashworth, K. A. Oien, T. R. Evans and O. J. Sansom (2010). "LKB1 haploinsufficiency cooperates with Kras to promote pancreatic cancer through suppression of p21-dependent growth arrest." Gastroenterology **139**(2): 586-97, 597 e1-6.*

*Nakada, D., T. L. Saunders and S. J. Morrison (2010). "Lkb1 regulates cell cycle and energy metabolism in haematopoietic stem cells." Nature **468**(7324): 653-8.*

*Nakanishi, C., T. Yamaguchi, T. Iijima, S. Saji, M. Toi, T. Mori and M. Miyaki (2004). "Germline mutation of the LKB1/STK11 gene with loss of the normal allele in an aggressive breast cancer of Peutz-Jeghers syndrome." Oncology **67**(5-6): 476-9.*

*Nakanishi, Y., M. Nakatsuji, H. Seno, S. Ishizu, R. Akitake-Kawano, K. Kanda, T. Ueo, H. Komekado, M. Kawada, M. Minami and T. Chiba (2011). "COX-2 inhibition alters the phenotype of tumor-associated macrophages from M2 to M1 in ApcMin/+ mouse polyps." Carcinogenesis **32**(9): 1333-9.*

*Nakau, M., H. Miyoshi, M. F. Seldin, M. Imamura, M. Oshima and M. M. Taketo (2002). "Hepatocellular carcinoma caused by loss of heterozygosity in Lkb1 gene knockout mice." Cancer Res **62**(16): 4549-53.*

*Noah, T. K., A. Kazanjian, J. Whitsett and N. F. Shroyer (2010). "SAM pointed domain ETS factor (SPDEF) regulates terminal differentiation and maturation of intestinal goblet cells." Exp Cell Res **316**(3): 452-65.*

*Ohashi, K., N. Ouchi, A. Higuchi, R. J. Shaw and K. Walsh (2010). "LKB1 deficiency in Tie2-Cre-expressing cells impairs ischemia-induced angiogenesis." J Biol Chem **285**(29): 22291-8.*

*Ohkusa, T., I. Takashimizu, K. Fujiki, S. Suzuki, K. Shimoi, T. Horiuchi, T. Sakurazawa, K. Ariake, K. Ishii, J. Kumagai and T. Tanizawa (1998). "Disappearance of hyperplastic polyps in the stomach after eradication of Helicobacter pylori. A randomized, clinical trial." Ann Intern Med **129**(9): 712-5.*

Ohmura, T., G. Shioi, M. Hirano and S. Aizawa (2012). "Neural tube defects by NUAK1 and NUAK2 double mutation." Dev Dyn.

*Orlova, K. A., W. E. Parker, G. G. Heuer, V. Tsai, J. Yoon, M. Baybis, R. S. Fenning, K. Strauss and P. B. Crino (2010). "STRADalpha deficiency results in aberrant mTORC1 signaling during corticogenesis in humans and mice." J Clin Invest **120**(5): 1591-602.*

*Pearson, H. B., A. McCarthy, C. M. Collins, A. Ashworth and A. R. Clarke (2008). "Lkb1 deficiency causes prostate neoplasia in the mouse." Cancer Res **68**(7): 2223-32.*

Peutz, J. L. A. (1921). "Very remarkable case of familial polyposis of mucous membrane of intestinal tract and nasopharynx accompanied by peculiar pigmentations of skin and mucous membrane. ." Nederl Maandschr Geneesk. (10): 134-146.

*Pham, H., B. Chong, R. Vincenti and L. W. Slice (2008). "Ang II and EGF synergistically induce COX-2 expression via CREB in intestinal epithelial cells." J Cell Physiol **214**(1): 96-109.*

*Phillips, R. K., M. H. Wallace, P. M. Lynch, E. Hawk, G. B. Gordon, B. P. Saunders, N. Wakabayashi, Y. Shen, S. Zimmerman, L. Godio, M. Rodrigues-Bigas, L. K. Su, J. Sherman, G. Kelloff, B. Levin and G. Steinbach (2002). "A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis." Gut **50**(6): 857-60.*

Pollak, M. N. (2012). "Investigating Metformin for Cancer Prevention and Treatment: The End of the Beginning." Cancer Discov.

*Pollard, P. J., J. J. Briere, N. A. Alam, J. Barwell, E. Barclay, N. C. Wortham, T. Hunt, M. Mitchell, S. Olpin, S. J. Moat, I. P. Hargreaves, S. J. Heales, Y. L. Chung, J. R. Griffiths, A. Dalgleish, J. A. McGrath, M. J. Gleeson, S. V. Hodgson, R. Poulsom, P. Rustin and I. P. Tomlinson (2005). "Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations." Hum Mol Genet **14**(15): 2231-9.*

*Puffenberger, E. G., K. A. Strauss, K. E. Ramsey, D. W. Craig, D. A. Stephan, D. L. Robinson, C. L. Hendrickson, S. Gottlieb, D. A. Ramsay, V. M. Siu, G. G. Heuer, P. B. Crino and D. H. Morton (2007). "Polyhydramnios, megalencephaly and symptomatic epilepsy caused by a homozygous 7-kilobase deletion in LYK5." Brain **130**(Pt 7): 1929-41.*

*Ratovitski, E. A. (2010). "LKB1/PEA3/DeltaNp63 pathway regulates PTGS-2 (COX-2) transcription in lung cancer cells upon cigarette smoke exposure." Oxid Med Cell Longev **3**(5): 317-24.*

*Rattan, R., S. Giri, A. K. Singh and I. Singh (2005). "5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside inhibits cancer cell proliferation in vitro and in vivo via AMP-activated protein kinase." J Biol Chem **280**(47): 39582-93.*

*Reig, I., P. Boixeda, B. Fleta, C. Morenoc, L. Gamez and M. Truchuelo (2011). "Neurofibromatosis-Noonan syndrome: case report and clinicopathogenic review of the Neurofibromatosis-Noonan syndrome and RAS-MAPK pathway." Dermatol Online J **17**(4): 4.*

Reyners, A. K., L. de Munck, F. L. Erdkamp, W. M. Smit, K. Hoekman, R. I. Lalisang, H. de Graaf, A. N. Wymenga, M. Polee, H. Hollema, M. A. van Vugt, M. Schaapveld and P. H. Willemse (2012). "A randomized phase II study investigating the addition of the specific COX-2 inhibitor celecoxib to docetaxel plus carboplatin as first-line chemotherapy for stage IC to IV epithelial ovarian cancer, Fallopian tube or primary peritoneal carcinomas: the DoCaCel study." Ann Oncol.

*Rintala, A. (1959). "The histological appearance of gastrointestinal polyps in the Peutz-Jeghers syndrome." Acta Chir Scand **117**: 366-73.*

*Robinson, J., C. Lai, A. Martin, E. Nye, I. Tomlinson and A. Silver (2009). "Oral rapamycin reduces tumour burden and vascularization in Lkb1(+/-) mice." J Pathol **219**(1): 35-40.*

*Robinson, J., E. Nye, G. Stamp and A. Silver (2008). "Osteogenic tumours in Lkb1-deficient mice." Exp Mol Pathol **85**(3): 223-6.*

*Romito, A., E. Lonardo, G. Roma, G. Minchiotti, A. Ballabio and G. Cobellis (2010). "Lack of sik1 in mouse embryonic stem cells impairs cardiomyogenesis by down-regulating the cyclin-dependent kinase inhibitor p57kip2." PLoS One **5**(2): e9029.*

*Rossi, D. J., A. Ylikorkala, N. Korsisaari, R. Salovaara, K. Luukko, V. Launonen, M. Henkemeyer, A. Ristimaki, L. A. Aaltonen and T. P. Makela (2002). "Induction of cyclooxygenase-2 in a mouse model of Peutz-Jeghers polyposis." Proc Natl Acad Sci U S A **99**(19): 12327-32.*

*Russell, R. R., 3rd, J. Li, D. L. Coven, M. Pypaert, C. Zechner, M. Palmeri, F. J. Giordano, J. Mu, M. J. Birnbaum and L. H. Young (2004). "AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury." J Clin Invest **114**(4): 495-503.*

*Sakamoto, K., O. Goransson, D. G. Hardie and D. R. Alessi (2004). "Activity of LKB1 and AMPK-related kinases in skeletal muscle: effects of contraction, phenformin, and AICAR." Am J Physiol Endocrinol Metab **287**(2): E310-7.*

*Sakamoto, K., A. McCarthy, D. Smith, K. A. Green, D. Grahame Hardie, A. Ashworth and D. R. Alessi (2005). "Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction." EMBO J **24**(10): 1810-20.*

*Sakamoto, K., E. Zarrinpashneh, G. R. Budas, A. C. Pouleur, A. Dutta, A. R. Prescott, J. L. Vanoverschelde, A. Ashworth, A. Jovanovic, D. R. Alessi and L. Bertrand (2006). "Deficiency of LKB1 in heart prevents ischemia-mediated activation of AMPKalpha2 but not AMPKalpha1." Am J Physiol Endocrinol Metab **290**(5): E780-8.*

*Sanchez, I., R. T. Hughes, B. J. Mayer, K. Yee, J. R. Woodgett, J. Avruch, J. M. Kyriakis and L. I. Zon (1994). "Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun." Nature **372**(6508): 794-8.*

*Sanchez-Cespedes, M., P. Parrella, M. Esteller, S. Nomoto, B. Trink, J. M. Engles, W. H. Westra, J. G. Herman and D. Sidransky (2002). "Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung." Cancer Res **62**(13): 3659-62.*

*Sapkota, G. P., M. Deak, A. Kieloch, N. Morrice, A. A. Goodarzi, C. Smythe, Y. Shiloh, S. P. Lees-Miller and D. R. Alessi (2002). "Ionizing radiation induces ataxia telangiectasia mutated kinase (ATM)-mediated phosphorylation of LKB1/STK11 at Thr-366." Biochem J **368**(Pt 2): 507-16.*

Sapkota, G. P., A. Kieloch, J. M. Lizcano, S. Lain, J. S. Arthur, M. R. Williams, N. Morrice, M. Deak and D. R. Alessi (2001). "Phosphorylation of the protein kinase mutated in Peutz-Jeghers cancer syndrome,

LKB1/STK11, at Ser431 by p90(RSK) and cAMP-dependent protein kinase, but not its farnesylation at Cys(433), is essential for LKB1 to suppress cell vrowth." J Biol Chem ***276****(22): 19469-82.*

Sasagawa, S., H. Takemori, T. Uebi, D. Ikegami, K. Hiramatsu, S. Ikegawa, H. Yoshikawa and N. Tsumaki (2012). "SIK3 is essential for chondrocyte hypertrophy during skeletal development in mice." Development ***139****(6): 1153-63.*

Sasaki, T., H. Takemori, Y. Yagita, Y. Terasaki, T. Uebi, N. Horike, H. Takagi, T. Susumu, H. Teraoka, K. Kusano, O. Hatano, N. Oyama, Y. Sugiyama, S. Sakoda and K. Kitagawa (2011). "SIK2 is a key regulator for neuronal survival after ischemia via TORC1-CREB." Neuron ***69****(1): 106-19.*

Sato, N., C. Rosty, M. Jansen, N. Fukushima, T. Ueki, C. J. Yeo, J. L. Cameron, C. A. Iacobuzio-Donahue, R. H. Hruban and M. Goggins (2001). "STK11/LKB1 Peutz-Jeghers gene inactivation in intraductal papillary-mucinous neoplasms of the pancreas." Am J Pathol ***159****(6): 2017-22.*

Sato, T., K. Yoshinaga, S. Okabe, T. Okawa, T. Higuchi, M. Enomoto, T. Takizawa and K. Sugihara (2003). "Cyclooxygenase-2 expression and its relationship with proliferation of colorectal adenomas." Jpn J Clin Oncol ***33****(12): 631-5.*

Seno, H., M. Oshima, T. O. Ishikawa, H. Oshima, K. Takaku, T. Chiba, S. Narumiya and M. M. Taketo (2002). "Cyclooxygenase 2- and prostaglandin E(2) receptor EP(2)-dependent angiogenesis in Apc(Delta716) mouse intestinal polyps." Cancer Res ***62****(2): 506-11.*

Shackelford, D. B. and R. J. Shaw (2009). "The LKB1-AMPK pathway: metabolism and growth control in tumour suppression." Nat Rev Cancer ***9****(8): 563-75.*

Shackelford, D. B., D. S. Vasquez, J. Corbeil, S. Wu, M. Leblanc, C. L. Wu, D. R. Vera and R. J. Shaw (2009). "mTOR and HIF-1alpha-mediated tumor metabolism in an LKB1 mouse model of Peutz-Jeghers syndrome." Proc Natl Acad Sci U S A ***106****(27): 11137-42.*

Shaw, R. J., N. Bardeesy, B. D. Manning, L. Lopez, M. Kosmatka, R. A. DePinho and L. C. Cantley (2004b). "The LKB1 tumor suppressor negatively regulates mTOR signaling." Cancer Cell ***6****(1): 91-9.*

Shaw, R. J., M. Kosmatka, N. Bardeesy, R. L. Hurley, L. A. Witters, R. A. DePinho and L. C. Cantley (2004a). "The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress." Proc Natl Acad Sci U S A ***101****(10): 3329-35.*

Shaw, R. J., K. A. Lamia, D. Vasquez, S. H. Koo, N. Bardeesy, R. A. Depinho, M. Montminy and L. C. Cantley (2005). "The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin." Science ***310****(5754): 1642-6.*

Shen, Z., X. F. Wen, F. Lan, Z. Z. Shen and Z. M. Shao (2002). "The tumor suppressor gene LKB1 is associated with prognosis in human breast carcinoma." Clin Cancer Res ***8****(7): 2085-90.*

Shorning, B. Y., D. Griffiths and A. R. Clarke (2011). "Lkb1 and Pten synergise to suppress mTOR-mediated tumorigenesis and epithelial-mesenchymal transition in the mouse bladder." PLoS One ***6****(1): e16209.*

Shorning, B. Y., T. Jarde, A. McCarthy, A. Ashworth, W. W. de Leng, G. J. Offerhaus, N. Resta, T. Dale and A. R. Clarke (2012). "Intestinal renin-angiotensin system is stimulated after deletion of Lkb1." Gut ***61****(2): 202-13.*

Shorning, B. Y., J. Zabkiewicz, A. McCarthy, H. B. Pearson, D. J. Winton, O. J. Sansom, A. Ashworth and A. R. Clarke (2009). "Lkb1 deficiency alters goblet and paneth cell differentiation in the small intestine." PLoS One ***4****(1): e4264.*

Sinicrope, F. A., E. Half, J. S. Morris, P. M. Lynch, J. D. Morrow, B. Levin, E. T. Hawk, D. S. Cohen, G. D. Ayers and L. C. Stephens (2004). "Cell proliferation and apoptotic indices predict adenoma regression

*in a placebo-controlled trial of celecoxib in familial adenomatous polyposis patients." Cancer Epidemiol Biomarkers Prev **13**(6): 920-7.*

*Smith, C. D., R. A. Compton, J. S. Bowler, J. T. Kemp, S. N. Sudweeks, D. M. Thomson and W. W. Winder (2011). "Characterization of the liver kinase B1-mouse protein-25 -Ste-20-related adaptor protein complex in adult mouse skeletal muscle." J Appl Physiol **111**(6): 1622-8.*

*Sommerhaug, R. G. and T. Mason (1970). "Peutz-Jeghers syndrome and ureteral polyposis." JAMA **211**(1): 120-2.*

*Steinbach, G., P. M. Lynch, R. K. Phillips, M. H. Wallace, E. Hawk, G. B. Gordon, N. Wakabayashi, B. Saunders, Y. Shen, T. Fujimura, L. K. Su and B. Levin (2000). "The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis." N Engl J Med **342**(26): 1946-52.*

*Su, G. H., R. H. Hruban, R. K. Bansal, G. S. Bova, D. J. Tang, M. C. Shekher, A. M. Westerman, M. M. Entius, M. Goggins, C. J. Yeo and S. E. Kern (1999). "Germline and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers." Am J Pathol **154**(6): 1835-40.*

*Sugden, C., R. M. Crawford, N. G. Halford and D. G. Hardie (1999). "Regulation of spinach SNF1-related (SnRK1) kinases by protein kinases and phosphatases is associated with phosphorylation of the T loop and is regulated by 5'-AMP." Plant J **19**(4): 433-9.*

*Sun, G., R. Reynolds, I. Leclerc and G. A. Rutter (2010a). "RIP2-mediated LKB1 deletion causes axon degeneration in the spinal cord and hind-limb paralysis." Dis Model Mech **4**(2): 193-202.*

*Sun, G., A. I. Tarasov, J. A. McGinty, P. M. French, A. McDonald, I. Leclerc and G. A. Rutter (2010b). "LKB1 deletion with the RIP2.Cre transgene modifies pancreatic beta-cell morphology and enhances insulin secretion in vivo." Am J Physiol Endocrinol Metab **298**(6): E1261-73.*

*Takeda, H., H. Miyoshi, Y. Kojima, M. Oshima and M. M. Taketo (2006). "Accelerated onsets of gastric hamartomas and hepatic adenomas/carcinomas in Lkb1+/-p53-/- compound mutant mice." Oncogene **25**(12): 1816-20.*

*Takeda, H., H. Miyoshi, Y. Tamai, M. Oshima and M. M. Taketo (2004). "Simultaneous expression of COX-2 and mPGES-1 in mouse gastrointestinal hamartomas." Br J Cancer **90**(3): 701-4.*

*Takeda, H., M. Sonoshita, H. Oshima, K. Sugihara, P. C. Chulada, R. Langenbach, M. Oshima and M. M. Taketo (2003). "Cooperation of cyclooxygenase 1 and cyclooxygenase 2 in intestinal polyposis." Cancer Res **63**(16): 4872-7.*

*Tang, E. I., X. Xiao, D. D. Mruk, X. J. Qian, K. W. Mok, P. Jenardhanan, W. M. Lee, P. P. Mathur and C. Y. Cheng (2012). "Microtubule affinity-regulating kinase 4 (MARK4) is a component of the ectoplasmic specialization in the rat testis." Spermatogenesis **2**(2): 117-126.*

*Tanwar, P. S., T. Kaneko-Tarui, L. Zhang, Y. Tanaka, C. P. Crum and J. M. Teixeira (2012). "Stromal Liver Kinase B1 [STK11] Signaling Loss Induces Oviductal Adenomas and Endometrial Cancer by Activating Mammalian Target of Rapamycin Complex 1." PLoS Genet **8**(8): e1002906.*

*Thomson, D. M., J. D. Brown, N. Fillmore, B. M. Condon, H. J. Kim, J. R. Barrow and W. W. Winder (2007a). "LKB1 and the regulation of malonyl-CoA and fatty acid oxidation in muscle." Am J Physiol Endocrinol Metab **293**(6): E1572-9.*

*Thomson, D. M., C. R. Hancock, B. G. Evanson, S. G. Kenney, B. B. Malan, A. D. Mongillo, J. D. Brown, S. Hepworth, N. Fillmore, A. C. Parcell, D. L. Kooyman and W. W. Winder (2010). "Skeletal muscle dysfunction in muscle-specific LKB1 knockout mice." J Appl Physiol **108**(6): 1775-85.*

*Thomson, D. M., B. B. Porter, J. H. Tall, H. J. Kim, J. R. Barrow and W. W. Winder (2007b). "Skeletal muscle and heart LKB1 deficiency causes decreased voluntary running and reduced muscle mitochondrial marker enzyme expression in mice." Am J Physiol Endocrinol Metab **292**(1): E196-202.*

*Tiainen, M., K. Vaahtomeri, A. Ylikorkala and T. P. Makela (2002). "Growth arrest by the LKB1 tumor suppressor: induction of p21(WAF1/CIP1)." Hum Mol Genet **11**(13): 1497-504.*

*Tobi, M. (1999). "Polyps as biomarkers for colorectal neoplasia." Front Biosci **4**: D329-38.*

*Towler, M. C., S. Fogarty, S. A. Hawley, D. A. Pan, D. M. Martin, N. A. Morrice, A. McCarthy, M. N. Galardo, S. B. Meroni, S. B. Cigorraga, A. Ashworth, K. Sakamoto and D. G. Hardie (2008). "A novel short splice variant of the tumour suppressor LKB1 is required for spermiogenesis." Biochem J **416**(1): 1-14.*

*Udd, L., P. Katajisto, D. J. Rossi, A. Lepisto, A. M. Lahesmaa, A. Ylikorkala, H. J. Jarvinen, A. P. Ristimaki and T. P. Makela (2004). "Suppression of Peutz-Jeghers polyposis by inhibition of cyclooxygenase-2." Gastroenterology **127**(4): 1030-7.*

*Udd, L. and T. P. Makela (2011). "LKB1 signaling in advancing cell differentiation." Fam Cancer **10**(3): 425-35.*

*Uebi, T., Y. Itoh, O. Hatano, A. Kumagai, M. Sanosaka, T. Sasaki, S. Sasagawa, J. Doi, K. Tatsumi, K. Mitamura, E. Morii, K. Aozasa, T. Kawamura, M. Okumura, J. Nakae, H. Takikawa, T. Fukusato, M. Koura, M. Nish, A. Hamsten, A. Silveira, A. M. Bertorello, K. Kitagawa, Y. Nagaoka, H. Kawahara, T. Tomonaga, T. Naka, S. Ikegawa, N. Tsumaki, J. Matsuda and H. Takemori (2012). "Involvement of SIK3 in Glucose and Lipid Homeostasis in Mice." PLoS One **7**(5): e37803.*

*Upadhyay, S., C. Liu, A. Chatterjee, M. O. Hoque, M. S. Kim, J. Engles, W. Westra, B. Trink, E. Ratovitski and D. Sidransky (2006). "LKB1/STK11 suppresses cyclooxygenase-2 induction and cellular invasion through PEA3 in lung cancer." Cancer Res **66**(16): 7870-9.*

*Vaahtomeri, K., E. Ventela, K. Laajanen, P. Katajisto, P. J. Wipff, B. Hinz, T. Vallenius, M. Tiainen and T. P. Makela (2008). "Lkb1 is required for TGFbeta-mediated myofibroblast differentiation." J Cell Sci **121**(Pt 21): 3531-40.*

*Vallenius, T., K. Vaahtomeri, B. Kovac, A. M. Osiceanu, M. Viljanen and T. P. Makela (2011). "An association between NUAK2 and MRIP reveals a novel mechanism for regulation of actin stress fibers." J Cell Sci **124**(Pt 3): 384-93.*

*van der Velden, Y. U., L. Wang, J. Zevenhoven, E. van Rooijen, M. van Lohuizen, R. H. Giles, H. Clevers and A. P. Haramis (2011). "The serine-threonine kinase LKB1 is essential for survival under energetic stress in zebrafish." Proc Natl Acad Sci U S A **108**(11): 4358-63.*

*van Lier, M. G., A. M. Westerman, A. Wagner, C. W. Looman, J. H. Wilson, F. W. de Rooij, V. E. Lemmens, E. J. Kuipers, E. M. Mathus-Vliegen and M. E. van Leerdam (2011). "High cancer risk and increased mortality in patients with Peutz-Jeghers syndrome." Gut **60**(2): 141-7.*

*van Veelen, W., S. E. Korsse, L. van de Laar and M. P. Peppelenbosch (2011). "The long and winding road to rational treatment of cancer associated with LKB1/AMPK/TSC/mTORC1 signaling." Oncogene **30**(20): 2289-303.*

*Vane, J. R., J. A. Mitchell, I. Appleton, A. Tomlinson, D. Bishop-Bailey, J. Croxtall and D. A. Willoughby (1994). "Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation." Proc Natl Acad Sci U S A **91**(6): 2046-50.*

Viollet, B., F. Andreelli, S. B. Jorgensen, C. Perrin, A. Geloen, D. Flamez, J. Mu, C. Lenzner, O. Baud, M. Bennoun, E. Gomas, G. Nicolas, J. F. Wojtaszewski, A. Kahn, D. Carling, F. C. Schuit, M. J. Birnbaum, E.

*A. Richter, R. Burcelin and S. Vaulont (2003). "The AMP-activated protein kinase alpha2 catalytic subunit controls whole-body insulin sensitivity." J Clin Invest **111**(1): 91-8.*

*Viollet, B., Y. Athea, R. Mounier, B. Guigas, E. Zarrinpashneh, S. Horman, L. Lantier, S. Hebrard, J. Devin-Leclerc, C. Beauloye, M. Foretz, F. Andreelli, R. Ventura-Clapier and L. Bertrand (2009a). "AMPK: Lessons from transgenic and knockout animals." Front Biosci **14**: 19-44.*

*Viollet, B., L. Lantier, J. Devin-Leclerc, S. Hebrard, C. Amouyal, R. Mounier, M. Foretz and F. Andreelli (2009b). "Targeting the AMPK pathway for the treatment of Type 2 diabetes." Front Biosci **14**: 3380-400.*

*Volikos, E., J. Robinson, K. Aittomaki, J. P. Mecklin, H. Jarvinen, A. M. Westerman, F. W. de Rooji, T. Vogel, G. Moeslein, V. Launonen, I. P. Tomlinson, A. R. Silver and L. A. Aaltonen (2006). "LKB1 exonic and whole gene deletions are a common cause of Peutz-Jeghers syndrome." J Med Genet **43**(5): e18.*

*Wada, K., M. Tanaka and K. Yamaguchi (1987). "Carcinoma and polyps of the gallbladder associated with Peutz-Jeghers syndrome." Dig Dis Sci **32**(8): 943-6.*

*Wang, Z. J., I. Ellis, P. Zauber, T. Iwama, C. Marchese, I. Talbot, W. H. Xue, Z. Y. Yan and I. Tomlinson (1999). "Allelic imbalance at the LKB1 (STK11) locus in tumours from patients with Peutz-Jeghers' syndrome provides evidence for a hamartoma-(adenoma)-carcinoma sequence." J Pathol **188**(1): 9-13.*

*Wei, C., C. I. Amos, A. Rashid, M. Sabripour, L. Nations, T. J. McGarrity and M. L. Frazier (2003). "Correlation of staining for LKB1 and COX-2 in hamartomatous polyps and carcinomas from patients with Peutz-Jeghers syndrome." J Histochem Cytochem **51**(12): 1665-72.*

*Wei, C., C. I. Amos, L. C. Stephens, I. Campos, J. M. Deng, R. R. Behringer, A. Rashid and M. L. Frazier (2005). "Mutation of Lkb1 and p53 genes exert a cooperative effect on tumorigenesis." Cancer Res **65**(24): 11297-303.*

*Wei, C., C. I. Amos, N. Zhang, X. Wang, A. Rashid, C. L. Walker, R. R. Behringer and M. L. Frazier (2008). "Suppression of Peutz-Jeghers polyposis by targeting mammalian target of rapamycin signaling." Clin Cancer Res **14**(4): 1167-71.*

*Wei, C., C. I. Amos, N. Zhang, J. Zhu, X. Wang and M. L. Frazier (2009). "Chemopreventive efficacy of rapamycin on Peutz-Jeghers syndrome in a mouse model." Cancer Lett **277**(2): 149-54.*

*Wera, S. (1999). "An EXCEL-based method to search for potential Ser/Thr-phosphorylation sites in proteins." Comput Methods Programs Biomed **58**(1): 65-8.*

*Wingo, S. N., T. D. Gallardo, E. A. Akbay, M. C. Liang, C. M. Contreras, T. Boren, T. Shimamura, D. S. Miller, N. E. Sharpless, N. Bardeesy, D. J. Kwiatkowski, J. O. Schorge, K. K. Wong and D. H. Castrillon (2009). "Somatic LKB1 mutations promote cervical cancer progression." PLoS One **4**(4): e5137.*

*Woodford-Richens, K., J. Williamson, S. Bevan, J. Young, B. Leggett, I. Frayling, Y. Thway, S. Hodgson, J. C. Kim, T. Iwama, M. Novelli, D. Sheer, R. Poulsom, N. Wright, R. Houlston and I. Tomlinson (2000). "Allelic loss at SMAD4 in polyps from juvenile polyposis patients and use of fluorescence in situ hybridization to demonstrate clonal origin of the epithelium." Cancer Res **60**(9): 2477-82.*

*Woodford-Richens, K. L., S. Halford, A. Rowan, S. Bevan, L. A. Aaltonen, H. Wasan, D. Bicknell, W. F. Bodmer, R. S. Houlston and I. P. Tomlinson (2001). "CDX2 mutations do not account for juvenile polyposis or Peutz-Jeghers syndrome and occur infrequently in sporadic colorectal cancers." Br J Cancer **84**(10): 1314-6.*

*Woods, A., A. J. Heslegrave, P. J. Muckett, A. P. Levene, M. Clements, M. Mobberley, T. A. Ryder, S. Abu-Hayyeh, C. Williamson, R. D. Goldin, A. Ashworth, D. J. Withers and D. Carling (2011). "LKB1 is required for hepatic bile acid transport and canalicular membrane integrity in mice." Biochem J **434**(1): 49-60.*

*Wullschleger, S., J. M. Garcia-Martinez and S. L. Duce (2012). "Quantitative MRI establishes the efficacy of PI3K inhibitor (GDC-0941) multi-treatments in PTEN-deficient mice lymphoma." Anticancer Res **32**(2): 415-20.*

*Xiao, B., M. J. Sanders, E. Underwood, R. Heath, F. V. Mayer, D. Carmena, C. Jing, P. A. Walker, J. F. Eccleston, L. F. Haire, P. Saiu, S. A. Howell, R. Aasland, S. R. Martin, D. Carling and S. J. Gamblin (2011). "Structure of mammalian AMPK and its regulation by ADP." Nature **472**(7342): 230-3.*

*Xie, Z., Y. Dong, J. Zhang, R. Scholz, D. Neumann and M. H. Zou (2009). "Identification of the serine 307 of LKB1 as a novel phosphorylation site essential for its nucleocytoplasmic transport and endothelial cell angiogenesis." Mol Cell Biol **29**(13): 3582-96.*

*Yamamoto, M., C. Furihata, Y. Fujimitsu, T. Imai, K. Inada, H. Nakanishi and M. Tatematsu (1997). "Dose-dependent induction of both pepsinogen-altered pyloric glands and adenocarcinomas in the glandular stomach of C3H mice treated with N-methyl-N-nitrosourea." Jpn J Cancer Res **88**(3): 238-44.*

*Yang, Q., N. A. Bermingham, M. J. Finegold and H. Y. Zoghbi (2001). "Requirement of Math1 for secretory cell lineage commitment in the mouse intestine." Science **294**(5549): 2155-8.*

*Yang, T. L., Y. R. Su, C. S. Huang, J. C. Yu, Y. L. Lo, P. E. Wu and C. Y. Shen (2004). "High-resolution 19p13.2-13.3 allelotyping of breast carcinomas demonstrates frequent loss of heterozygosity." Genes Chromosomes Cancer **41**(3): 250-6.*

*Ylikorkala, A., D. J. Rossi, N. Korsisaari, K. Luukko, K. Alitalo, M. Henkemeyer and T. P. Makela (2001). "Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice." Science **293**(5533): 1323-6.*

*Yoo, L. I., D. C. Chung and J. Yuan (2002). "LKB1--a master tumour suppressor of the small intestine and beyond." Nat Rev Cancer **2**(7): 529-35.*

*Young, S. D., R. M. Lafrenie and M. J. Clemons (2012). "Phase ii trial of a metronomic schedule of docetaxel and capecitabine with concurrent celecoxib in patients with prior anthracycline exposure for metastatic breast cancer." Curr Oncol **19**(2): e75-83.*

*Zacksenhaus, E., Z. Jiang, D. Chung, J. D. Marth, R. A. Phillips and B. L. Gallie (1996). "pRb controls proliferation, differentiation, and death of skeletal muscle cells and other lineages during embryogenesis." Genes Dev **10**(23): 3051-64.*

*Zeqiraj, E., B. M. Filippi, M. Deak, D. R. Alessi and D. M. van Aalten (2009b). "Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation." Science **326**(5960): 1707-11.*

*Zeqiraj, E., B. M. Filippi, S. Goldie, I. Navratilova, J. Boudeau, M. Deak, D. R. Alessi and D. M. van Aalten (2009a). "ATP and MO25alpha regulate the conformational state of the STRADalpha pseudokinase and activation of the LKB1 tumour suppressor." PLoS Biol **7**(6): e1000126.*